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THE VASCULAR MECHANISM OF THE SPLEEN *

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The study of the pathologic processes in the spleen has been handicapped by an inadequate understanding of the histologic structure of this organ. A perusal of textbooks on histology aids very little. A review of the literature shows that much has been done and many points concerning its vascular system elucidated; but there are many details of structure which are not clear. The question of an open or closed circulation appears to be still unsettled.

This study was undertaken with the hope of obtaining more minute detail of structure and a better understanding of the blood flow through the spleen. In view of the complexity of the subject it is impossible to cover all its phases in one short paper. We are therefore confining our study more particularly to the distribution of the arteries, their relation to the pulp and the nature of the blood flow through this organ.

REVIEW OF LITERATURE

The spleen is a highly vascular organ through which passes a fairly constant flow of blood. This Mall [1] found in the dog to be approximately 5 cc. per minute. Its chief function apparently has to do with purification of the blood and the conversion of hemoglobin. Because of its peculiar anatomy, greatest attention has been paid to its vascular system and the relation of this to the parenchyma. It would appear, however, that the subordinate structures, such as capsule, trabeculae and stroma, are not entirely inert tissues, but by virtue of their muscle and elastic fibers, play an important rôle in

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assisting or impeding the flow of blood through this organ. The peculiar arrangement of the vascular system in its relation to the parenchyma as well as the supporting tissue necessitates, for a proper understanding of the vascular mechanism of the spleen, a study of each portion in detail.

Following death or upon extirpation, the spleen immediately contracts. Barcroft [2] has shown in the cat that following death from drowning or hemorrhage, it may shrink as much as one-half to one-sixth of its original size. For this reason, most experimenters have found it necessary to distend the pulp of the spleen by injections of fixing fluid through the artery or vein in order to demonstrate its histologic features.

Mall,[3] by a study of microscopic sections and macerated specimens of dog spleens, found it to have a capsule made up of connective tissue with muscle and elastic fibers. From the capsule he found prolongations extending into the splenic pulp, forming trabeculae and roughly dividing it into lobules of approximately 1 mm. in diameter. Prolongations from these consisting of muscle fibers alone, divided the lobule still further into smaller compartments. The larger interlobular trabeculae he found intimately associated with veins and so arranged as to pull them open when the trabeculae contract and compress the pulp.

Many workers have described the structure of the splenic pulp. It has been generally recognized as a cavernous, venous network, different in structure from that of any other organ. Leydig (quoted by Billroth),[4] Ludwig Stieda,[5] and others described the cavernous nature of the pulp with its network of star-like cells. Schweigger-Seidel,[6] on the other hand, denied the existence of such a vascular network, claiming that the pulp consisted only of a connective tissue framework supporting the vascular channels and filled with lymph. Oppel,[7] using Golgi's method, demonstrated a network of fibrils in the pulp, which Mall [3] believed were the reticulum fibers. These fibers Mall [8] believed were the supporting framework for the vascular system.

The peculiar structure of the splenic vein wall was first described by Malpighii [9] who noted the cribriform arrangement of their lining cells bound together by fibrils leaving slit-like stomas in their wall. Mall [3] showed that it was only the intralobular veins that had this peculiar type of wall. The large veins embedded in the trabeculae

he found had closed walls. The intralobular veins which were of a cribriform structure, he believed formed a closed system of intercommunicating channels; the veins were widely separated from the arteries of the same order. The fibrils binding the lining cells of the veins together, were believed by most experimenters to be reticulum. V. Ebner[10] thought they were elastic fibrils. Weidenreich[11] later showed they were protoplasmic processes of the pulp cells.

The distribution of the arteries in the spleen was early described by Malpighii[9] who found that the larger branches were clothed at irregular intervals with a lymphoid sheath. Kyber[12] by arterial injection showed that the branches penetrated the lymphoid sheath to break up into a number of non-anastamosing, finer branches in the pulp. What was thought to be the termination of these slender arterial twigs was described by Schweigger-Seidel[6] as "pear-shaped, globoid masses of cells enveloping the arteriole." He called them *Kapillarhülsen*. The term ellipsoid, however, has come to be more generally used to indicate these bodies. Billroth[13] noticed them first in the spleen of the bird, but failed to interpret their function and subsequently failed to attach any importance to them. Schweigger-Seidel studied them in the spleens of sheep, dog, cat and pig. After much diligent search he thought he found one in the human spleen. While in some cases he thought the arterioles ended blindly in the ellipsoid, in other cases he found that they apparently passed through. He thought that there were interstices in the ellipsoids other than the central capillaries, and believed that they were of the nature of a filter apparatus allowing only fluids to pass through. He did not believe that all the arterioles ended in this manner. Some, he thought, ended in other ways and besides there appeared to be too few of them to accommodate all the blood which passed through the spleen. Later Golz,[14] a co-worker of Thoma, using a granular injection mass, showed that the arterioles passed through the ellipsoids and ended in ampullatous dilatations of the vessels. These findings were confirmed by Mall[8] who designated the dilatation of the capillary as "the ampulla of Thoma." Thoma[15] believed that a direct communication was established between the ampullae and the neighboring veins by small communicating vessels, *Zwischenstück*, thus establishing a closed circulation through the spleen. Weidenreich[11] also believed that the arterioles passed through the ellipsoids and in some cases united directly with the

venous channels. In most cases, however, they appeared to break up and fuse with the pulp cells. Mall [3] believed that the ellipsoids were merely an extension of the lymphoid sheath of the Malpighian corpuscles along the arteriole branches. The ampulla of Thoma he divided into three parts: the first third had a distinct wall composed of spindle-shaped endothelial cells; the second third, the ampulla proper, was the dilated portion and had an irregular wall with openings into the pulp; the last third formed a communication with the veins. He was uncertain concerning the nature of this portion. In fact, he was not able to trace a continuous cell boundary from it to the veins, nor was he able to inject this portion of the ampulla from the venous side. Later he [1] came to the conclusion that the ampullae were merely large openings within the spongy pulp spaces — exaggerated pulp spaces.

The nature of the blood flow through the spleen has been the subject of much experimental discussion and study. Poelman [16] discovered that while colored fluid injected into the artery returned by the vein, the reverse was not true. It was impossible, he found, to pass fluid from the venous to the arteriole side. He thought that the arteries terminated in two ways, first by anastamotic arches, and second by an abrupt termination in labyrinthiform cavities. These latter he considered to be merely dilatations of the splenic vein. Poelman therefore must have looked upon the circulation through the spleen as a closed one. Billroth [13] believed that the arteries ended abruptly in the spleen and that the red blood cells after extravasation into the pulp spaces were pressed into the venous sinuses. However, he believed that in some cases the flow was direct, through a closed system as in other parts of the body. Bannwarth [17] believed the ellipsoids and end capillaries opened directly into the pulp spaces. Weidenreich,[11] on the other hand, concluded that the circulation was both by way of the pulp sinuses and by directly communicating end capillaries to the venous sinuses. Janosik,[18] however, believed that the circulation was closed. Thoma,[15] who held similar views, maintained that the difference of opinion was based largely upon the difference in the methods of experiment and in the character of the injection materials used. He said that all who used nonsoluble pigment in granular form had obtained results similar to his, and demonstrated a closed circulation. He admitted that the ampullae were permeable to plasma, thus explaining why all workers

using soluble pigment injection masses, got results indicating an open circulation. He believed that while the plasma may percolate through the ampulla into the pulp and thus to the veins, the cellular constituents of the blood were retained within closed channels. Mall [8] at first accepted Thoma's conception of the vascular construction of the spleen and also his view of the blood flow through it. Later, however, after further study and experimentation, he [1] changed his opinion both as to the blood flow and also to some degree as to the vascular structure. He became convinced that the last third of the ampulla of Thoma was cut up by bridges of pulp cells and that the flow of blood was therefore from ampulla to pulp and thence to the vein. In other words he believed that there was an open circulation. In substantiation of this claim, he outlined four experiments which he considered convincing evidence.

Experimental Work

Since it would appear that the pulp is the important functional tissue of the spleen, we will not enter into a detailed description of this phase of the subject at the present time, but will reserve it for a further report at a future date. In our present study we are concerned rather with the distribution of the arterioles, the nature of their termination, their relation to the pulp and venous sinuses, and the nature of the blood flow through the spleen.

TECHNIC

To mark the course and outline of the arteries and their terminal branches a variety of injection material was used, such as celloidin mixtures (thick and thin), oil, hot lard, asphaltum in turpentine, pigment and gelatine solutions. Of these the most satisfactory was gelatine with or without pigment added. Very good injections of the arterial system were made with warm 25 per cent aqueous solutions of plain gelatine. In cases in which it was desired to know the fate of an intravascular injection of particulate matter, a 5 per cent solution of carmine powder in 15 per cent gelatine solution was used. The gelatine was added as a medium to hold the pigment *in situ* when the pulp was distended with a fixative.

Thoma insisted upon the use of a granular pigment in his injection fluid, not only to indicate the course taken by the red blood cells, but also to outline the arteriole channels. We felt, however, that there

was a distinct objection to its use as a substitute for red blood cells, as it was a particulate matter decidedly foreign to the spleen and readily removed by mechanical filtration. In view of the natural fluidity of the red blood cells as shown by Krogh,[19] warm gelatine seemed a much better substitute.

Sheep spleens were used at first, as any number of them could be procured fresh from the abattoir. These had to be removed very carefully from the body in order that the pulp may not be torn by pulling on the vessels. Later, dog, human, cat and chicken spleens were also used. Because of the prominence of their ellipsoids, the dog spleens were found to be the most satisfactory. The arteries were injected with varying quantities of gelatine solution. The quantity of solution used was governed by the size of the spleen and the extent to which the artery and the surrounding pulp was to be injected. It was our desire as a minimum to fill just the arterial system and from this, quantitatively to grade up the injection until the pulp and veins were completely filled, and from these specimens to follow the sequence of events. In some instances, a single intravenous injection of India ink was used, in others repeated injections of pyrrol blue.

The pressure required for injection of the arterial system varied somewhat depending upon the freshness of the spleen and the nature of the injection mass and its temperature and consistence. In the case of warm gelatine solution, if the spleen was injected immediately following death prior to the clotting of the blood, approximately 100 to 200 mm. of mercury were usually found sufficient. On completion of the injection the artery was tied and the organ immersed in cold water to hasten the setting of the gelatine. After allowing sufficient time for this to take place, the spleen was distended to capacity by injecting through the vein either Zenker's fluid with formalin or 10 per cent formalin solution alone. Blocks were taken from various parts of the injected spleen and embedded in paraffin and cut at from 10 to 20 microns. A variety of stains was used, such as Mallory's eosin and methylene blue, phosphotungstic acid-hematoxylin, analine blue, hematoxylin and eosin and Van Gieson's stain. For general purposes, the Van Gieson's stain was found to be the most satisfactory. If the gelatine-injected sections were stained well with iron hematoxylin and carefully differentiated in dilute liquor ferri chloridi, the gelatine would retain the hematoxylin pro-

viding the sections were counterstained for only a few seconds with picro-acid fuchsin mixture. On the other hand, the gelatine injection mass could be stained with the acid fuchsin by differentiating well and staining deeply with the picro-acid fuchsin mixture. The gelatine thus stained outlined clearly the course of the blood through the arteries and pulp sinuses. When carmine pigment was added to the injection mass, picric acid alone was used as a counterstain and the sections were well differentiated. Reticulum stain and elastic tissue stain were also used and silver nitrate injections tried.

RESULTS

We were able to confirm Mall as to the lobular structure of the spleen, divided as it is by trabeculae, consisting of muscle, elastic and connective tissue; also that the interlobular veins are closely associated with the trabeculae, while the arteries occupy a more central position in the lobule.

Pulp. By using a Zeiss binocular microscope with stereoscopic attachments to the oculars, we obtained a three dimension view of our section. We were thus able to see the pulp in perspective and to obtain an accurate idea of its structure. It is found to consist of a vast delicate network of star-like cells having long irregular protoplasmic processes running in all directions uniting one cell with the other and forming attachments to the supporting trabecular framework. Their nuclei are oval, well stained and reticulated. There is very little cytoplasm about the nucleus. The bulk of it extends out in a fine web-like manner and ends in long filamentous processes which fuse with those from other pulp cells. These same cells form a covering for the trabeculae and larger blood vessels. By intravital injections the pulp cells are found to be phagocytic for colloids and particulate matter and therefore reticulo-endothelial in type. With a reticulum stain we were not able to demonstrate a supporting framework for these cells. What appeared to be reticulum is merely the protoplasmic processes of the pulp cells. The pulp cells, thus loosely held together, form with their protoplasmic processes a sponge-like structure having variegated, tortuous, intercellular spaces communicating freely with each other. These pulp spaces vary in size from 0.005 to 0.02 mm. in diameter.

Arteries. The general course of the arteries is found as described by Mall,[8] showing branches penetrating the lymphoid sheath or

Malpighian corpuscle and spreading through the central portion of the lobule. Finer branches consisting of a flattened layer of endothelial cells and surrounded by a muscular layer are found uniformly distributed throughout the pulp and supported directly by the pulp cells. Compared with the veins, they are very much smaller and stouter. After running a course of 0.2 to 1 mm. they appear to end abruptly in globoid masses of cells. These we recognized as the ellipsoids of Schweigger-Seidel. With thicker sections the arterial branches are found to continue through the ellipsoids as a single layer of endothelial cells devoid of a muscle coat.*

Ellipsoids. The ellipsoids in the dog and cat spleens vary in length from 0.17 to 0.24 mm. They are pear-shaped and have a diameter varying from 0.08 to 0.034 mm. In the sheep and human spleens they are much smaller. They are uniformly distributed throughout the pulp, occupying fairly central positions in the "histologic unit," as described by Mall.[8] The arterioles enter at the blunt end, the exit being at the opposite or pointed end. In cases in which the capillary divides within the ellipsoid there is an extension of the ellipsoid cells along the continuing capillaries producing a bicornate or tricornate structure. The cells making up the ellipsoid are found rather closely packed about the capillary which, however, remains quite patent. Using a Zeiss binocular microscope with a stereoscopic attachment for the oculars, we were able to view the ellipsoids in three dimensions and to observe quite clearly their minute detail and structure. With the monocular microscope, one gets the impression that the ellipsoids are a compact mass of cells. With stereoscopic vision we find quite the contrary. The cells are apparently of the same type as the pulp cells, but have more cytoplasm about the nucleus and shorter protoplasmic processes. They are united one with the other by these short processes and are distributed evenly throughout the ellipsoid. We were able to identify intercellular spaces much the same as are seen in the pulp except that they are much smaller, varying in size from one-half to twice the diameter of a red blood cell. Along the margin of the ellipsoid these intercellular spaces communicate freely with the adjacent pulp

* At the recent meeting of The American Association of Pathologists and Bacteriologists, held at Albany, N. Y., April 1, 1926, in my discussion of this subject, an erroneous impression may have been given, namely, that the arterial capillary within the ellipsoid ends blindly.

spaces. The ellipsoid capillary is suspended in the center by the protoplasmic processes of the ellipsoid cells. With the intravital stain the ellipsoid cells are found to be phagocytic for colloid and particulate matter. They are, therefore, functionally of the same type as the pulp cells.

End Arteries. The arteriole enters the blunt end of the ellipsoid and, devoid of its muscle layer, is found to pass through the center as a vessel of capillary size composed of a single layer of endothelial cells. In many cases the capillary divides within the ellipsoid before proceeding into the pulp. The ellipsoid capillaries continue into the pulp as an end capillary for a variable distance up to approximately 0.1 mm. With the binocular microscope, we were able to identify small rounded stomas in the walls of these end capillaries. Toward the termination of the capillaries, their walls in some cases gradually expand in a funnel-shaped manner to blend quite intimately with the pulp. The integrity of the capillary is then soon lost in the cavernous network of pulp cells. In most cases, however, the end capillary terminates in an ampullatous dilatation. These we recognized as the "ampullae of Thoma." They are cone-shaped and have a rounded base. There are large openings in their walls communicating freely with the surrounding pulp spaces. In their first part they appear to have a definite wall made up of the continuing cells of the end capillaries. However, as the vessel expands to form the ampulla, the lining cells fuse quite intimately with the pulp cells and become a part of the pulp tissue. The wall of the rest of the ampulla therefore does not have a definite layer of lining cells, but consists of pulp cells. The ampulla is merely an exaggerated pulp space. In support of this view, we were able in many instances to find the end capillaries expanding and fusing with the pulp cells in a most diffuse and intimate manner without the formation of any cavitation of the pulp tissue. In no case were we able to identify a communicating vessel between the ampulla and vein.

Veins. Coursing through the pulp and supported directly by the pulp cells, are found the large venous sinuses described by Mall and others. They are chiefly in the peripheral portions of the lobule, intimately associated with and in many cases surrounded by the trabeculae. Their walls are well formed. The finer venous branches course freely throughout the whole lobule anastamosing one with the other to form a branching network of collecting channels intimately

associated with the pulp. Their association with the ellipsoids in many cases is quite intimate. At times they almost completely surround them. Their walls, in contradistinction to the larger veins, are incomplete in structure. They are made up of parallel rows of elongated endothelial cells loosely bound together by the protoplasmic processes of the neighboring pulp cells. Slit-like stomas are left between the cells, giving it the appearance of a barrel with alternate staves removed. We were unable to demonstrate any other covering to these vessels. The nuclei of the lining cells are oval and bulge into the lumen. With intravital stains, they are found to be non-phagocytic.

Blood Flow. To demonstrate the blood flow through the spleen, varying quantities of warm gelatine solution with or without carmine pigment were injected into the arteries and veins. Sections were then examined. When a large amount of gelatine is injected into the artery it can be traced through the whole arterial system. The arteries and their branches up to the ellipsoids are found to be impermeable to the gelatine solution. However, it filters through the ellipsoid and appears in fine threads running out in all directions into the surrounding pulp spaces. Quite often the gelatine can be traced directly from the ellipsoid and through the wall of an adjacent venous sinus into its lumen. At other times the venous system is reached only after a devious course through the pulp tissue. Some of the gelatine can be traced on into the end capillaries and ampullae. Here again the permeability of these is manifested. Gelatine threads can be traced through the walls of the end capillaries and ampullae into the surrounding pulp spaces. When minimal amounts are injected and the arterial system alone filled, the arteries and their branches up to the ellipsoid again are impermeable to the injection mass. Beyond this their permeability varies. With minimal injection most of the gelatine passes on through the end capillaries into the ampullae. Here it scatters in all directions in the pulp spaces in an arborescent manner. In places the gelatine oozes through the walls of some of the end capillaries before they expand to form the ampullae.

The arterial terminations are less permeable for carmine pigment; some of it is filtered out by the ellipsoid and here appears in compact masses in the ellipsoid capillaries. Where an intravital injection of

India ink is made, the same filtrative phenomenon is manifested by the ellipsoid. Most of the carbon particles are held in the ellipsoid itself. Some of the granules, however, pass through into the adjacent pulp.

The injection mass, once having reached the pulp, appears no longer to be confined to channels. It is found to have spread out in all directions in a centrifugal manner as fine, filamentous threads. There is no general directional tendency nor any evidence of impediment to flow manifested by undue accumulation of the mass at any one point. Where the injection mass is sufficient, it can be traced by various routes from arterial to venous channels. Where small amounts are used, the bulk of the gelatine passes through the ellipsoid into the end capillaries and the ampullae and from here scatters out into the pulp spaces. In some cases, threads of gelatine can be traced through the tortuous pulp spaces into neighboring venous sinuses.

When the gelatine solution is injected into the venous system, we find that on reaching the intralobular veins it appears to have flowed out in a massive manner into the surrounding pulp spaces. These spaces adjoining the veins are completely filled with the gelatine solution. Very little pressure is required for these injections.

Discussion

After making a careful study of various animal spleens whose vascular system had been injected with gelatine and pigment solutions and the pulp distended to capacity with fixative, we believe that we have been able to demonstrate some of the minute histologic structures of this organ. While its lymphoid elements are important, the essential tissues are the pulp and ellipsoids. The pulp cells by their long, protoplasmic processes, are woven into a lace-like net to form a vast cavernous system of intercellular spaces freely communicating with one another. The cells are thus exposed on all surfaces to the content of the pulp spaces. The pulp and ellipsoid cells, being phagocytic for colloid and particulate matter, are endothelial in function. They are active phagocytic clasmatocytes which Aschoff [20] called reticulo-endothelial cells.

The capsule and trabeculae, besides acting as a supporting frame-

work, in a very large measure control the flow of blood through the pulp spaces. Mall,[1] Tait [21] and others have shown that with the relaxation of the musculo-elastic framework (the capsule and trabeculae), the pulp spaces fill with blood. On contraction the spaces are readily emptied.

The arterial system of the spleen is of unique design. It is non-anastamotic and ends in the cavernous system of pulp spaces. Near the endings of the finer arterial branches the muscle coat of the vessel is replaced by a compact mass of cells, the ellipsoid. These are endothelial in type and function. A stereoscopic view of the ellipsoids shows that they are not only made up of cells of the same type, but also have the same arrangement as those of the pulp. Intercellular spaces can be seen, similar to those of the pulp, freely communicating one with the other and with the contiguous pulp spaces. The ellipsoid spaces, of course, are much smaller. One might therefore look upon the ellipsoids as merely condensed masses of pulp tissue. Hoyer's [22] conception of them as a protective mechanism to the arteriole against undue stress during pressure increases in the venous circulation or against bursting during high arterial pressure, we think is worthy of consideration.

The ellipsoids do not represent the end of the arterial system. The terminal capillaries pass through them into the looser pulp tissue beyond. Here the capillary opens out and fuses with the pulp cells. The ellipsoids while permeable to gelatine solutions are much less so for particulate matter such as carmine pigment. Because of their small intercellular spaces the ellipsoids may serve to filter out very fine materials from the blood which otherwise might pass through the larger spaces of the pulp.

The end capillary or portion beyond the ellipsoid was demonstrated to be permeable to gelatine and with stereoscopic vision we were able to confirm this by discovering small rounded stomas in their walls. Where the end capillaries fused with the pulp, it appeared in most cases to end in an ampullatous dilatation of the vessel. A stereoscopic view of their walls shows that the outer half of the ampulla is composed of pulp cells alone and is incomplete in structure. In many cases the end capillaries fused with the pulp more intimately and no ampulla was discernible. For these reasons we believe that the "ampulla of Thoma" is merely an exaggerated pulp space produced, no doubt, by the concentration of blood flow at this

point. We were unable to find communicating vessels between the ampulla and the vein. From a study of our gelatine-injected specimens, we believe that the end capillaries serve to scatter the blood stream more widely into the pulp system.

The veins are of such structure, size and distribution as to drain readily the whole cavernous system of the pulp in a minimum of time. The walls of the finer branches are incomplete, having slit-like stomas communicating freely with the pulp spaces in a most intimate manner.

By the foregoing means we have determined the nature of the blood flow through the spleen. We believe that the circulation through this organ is an open one. The terminations of the arterial system are such as to scatter the blood diffusely into the pulp. The veins, by virtue of their size and incomplete walls, allow for its rapid emptying. The amount of blood maintained within the pulp is largely controlled by the muscular system of the capsule and spleen.

Conclusion

The fibro-musculo-elastic capsule and trabeculae serve as a skeletal framework for the splenic pulp which it divides into lobules.

The pulp consists of a network of reticulo-endothelial cells whose protoplasmic processes unite with one another to form a labyrinthiform system of intercellular spaces through which the blood flows.

The arterial circulation opens into the pulp by the flaring out and fusing of the end capillaries with pulp cells.

The "ampullae of Thoma" are exaggerated pulp spaces.

The ellipsoids have the same structure and cellular content as the pulp except that they are more compact.

The intralobular veins have incomplete walls, having slit-like stomas which communicate freely with the pulp spaces.

The ellipsoids and end capillaries are permeable to gelatine solution.

Gelatine solutions injected into the artery pass through the pulp before reaching the veins, except where a venous channel lies contiguous with the ellipsoid.

In this latter case, the flow is through the interstices of the ellipsoid and stomas of the vein wall into the lumen of the vein.

There are no direct communicating closed channels between arteries and veins other than the pulp spaces.

The circulation of the spleen is an open one.

We are indebted to Dr. George Kastlin for his help in reviewing the literature and assistance in the experimental work; and to Professor Klotz for his kindly criticisms while the work was progressing.

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DESCRIPTION OF PLATES

PLATE 68

FIG. 1. Ellipsoids with end capillaries. The end capillaries in the upper and lower right hand corners terminate in an ampulla. The two in the upper left hand corner fuse with the pulp cells without the formation of an ampulla. Cross-sections of nine veins can be seen in this photograph.

FIG. 2. Two end capillaries showing their lining cells fusing with those of the pulp without the formation of ampullae.

FIG. 3. Gelatine oozing from the end capillary into the surrounding pulp spaces.

PLATE 69

FIG. 4. Intralobular vein supported directly by the pulp cells. This photograph illustrates the structure of the vein and also the character of the pulp cells.

FIG. 5. Gelatine oozing directly from ellipsoid into adjacent vein.

PLATE 70

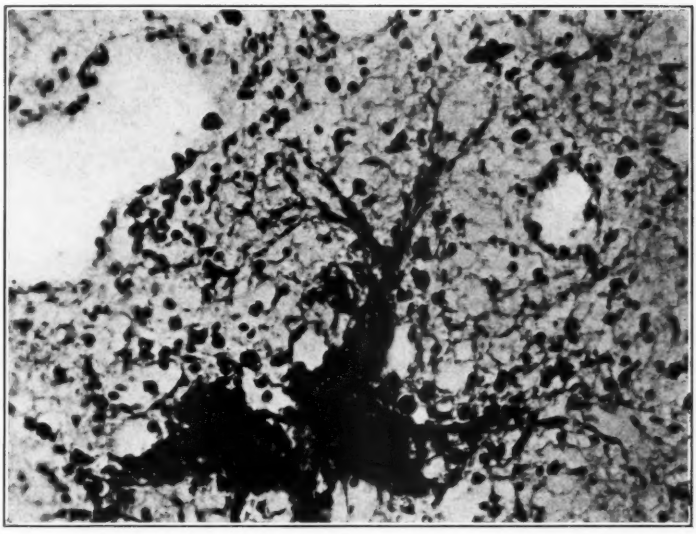
FIG. 6. Dog spleen showing pulp cells covering trabeculae.

FIG. 7. Human spleen. Branching ellipsoids.

FIG. 8. Sheep spleen. Gelatine oozing from vein into pulp.

FIG. 9. Sheep spleen. Gelatine oozing from ellipsoid into surrounding pulp spaces.

FIG. 10. Dog spleen, showing arteriole entering ellipsoid, passing through, and terminating in an ampulla.



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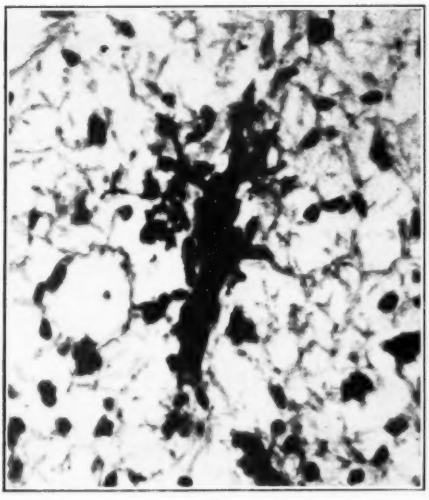

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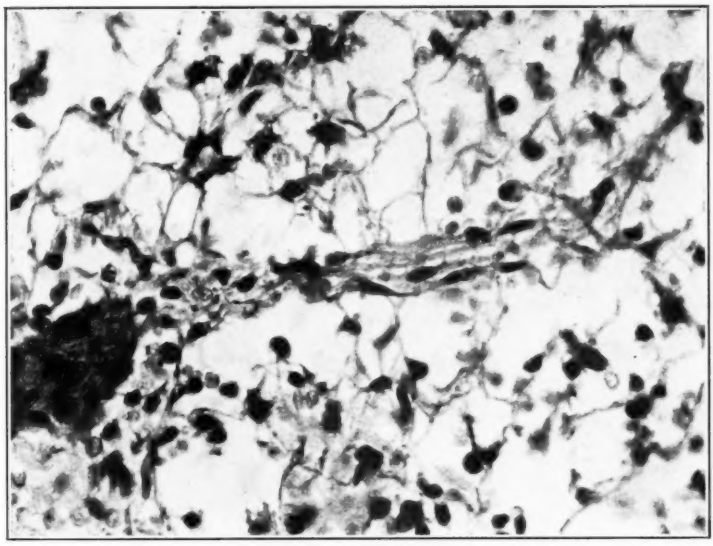
Robinson Vascular Mechanism of the Spleen



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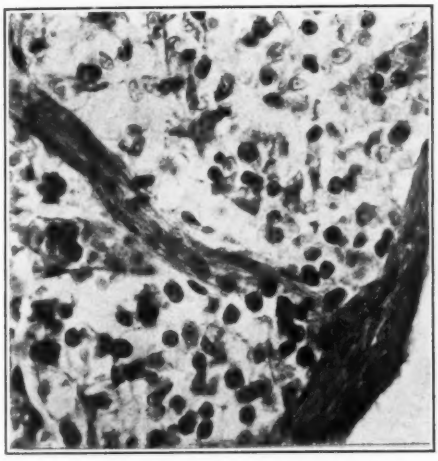
Robinson Vascular Mechanism of the Spleen



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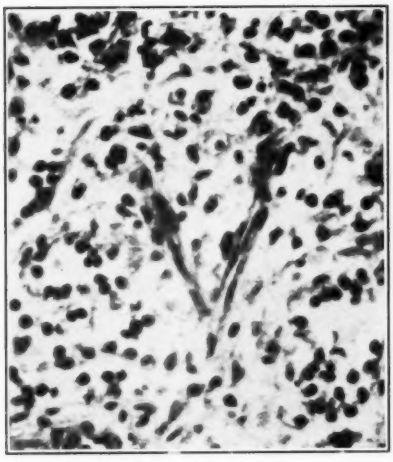

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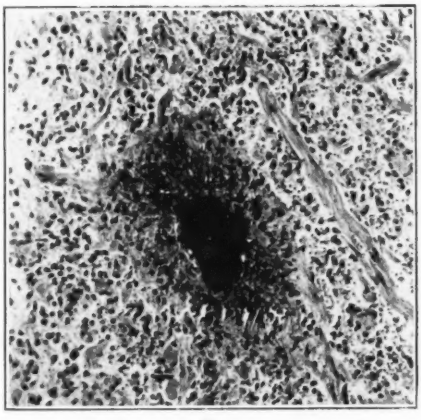

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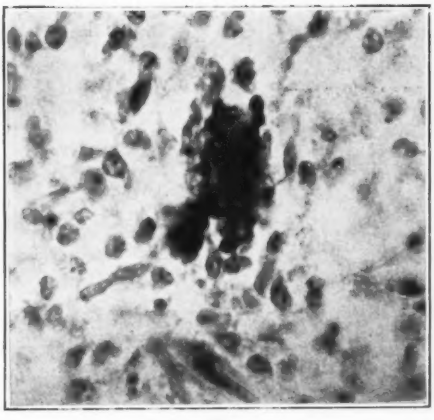

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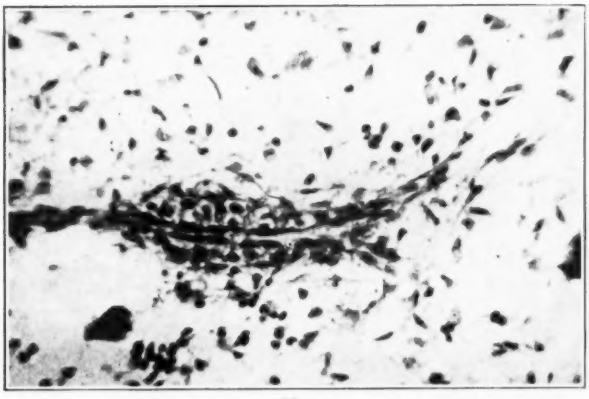

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THE ETIOLOGY OF HAVERHILL FEVER (ERYTHEMA ARTHRITICUM EPIDEMICUM) *

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INTRODUCTION

In a preliminary clinical report by Place, Sutton and Willner [1] of an epidemic that occurred in Haverhill, Mass., in January, 1926, we inserted a brief description of an organism we isolated from patients suffering with the disease. It is the purpose of this paper to give an account of the bacteriology and experimental pathology of this organism which has not previously been studied so far as we are able to determine.

Place and his collaborators have temporarily called the disease "Erythema Arthriticum Epidemicum," or Haverhill fever. They have summarized the clinical picture as being dominated "(1) by the acute onset with toxic symptoms, as chills, vomiting, malaise and headache, (2) the eruption, involving especially the extremities, of a blotchy somewhat morbilliform character with a tendency to petechiae and (3) by a multiple arthritis of varying but often severe degree." Epidemiologically, the disease was traced to raw milk distributed through stores by a farmer who handled, besides his own supply, milk from two small farms nearby.

At various stages of the disease, we made examinations of blood, throat, urine and joint fluid of patients. The blood of twenty-one patients was cultured, of which eleven were positive for the microorganism to be described (two positive twice, two positive once and negative twice, and one positive once and negative once); two were positive but contaminated and could not be purified; five were sterile; one was overheated in incubating; and four were contaminated, but apparently negative for the organism found in the positive cultures. Numerous throat cultures showed no unusual organisms. Urine cultures of three patients were too contaminated for further examination. Taps of swollen knee joints of two patients of whom one had positive blood cultures, gave pure cultures of the organism.

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These examinations furnished thirteen strains of which one was lost soon after recovery, leaving twelve for study, eleven from the blood and one from the knee joint. These strains are now being carried and have agreed in all respects.

Samples of milk from the twenty suspected cows on the three farms incriminated epidemiologically were cultured at the onset of the epidemic, but showed only the normal flora so far as could be determined at that time.

Numerous controls on the mediums used, technic of taking blood cultures and other sources of contamination support the reliability of the source of the organism. We believe that the microörganism recovered is the specific etiologic agent of the disease because of its high incidence in pure culture in the blood and knee joint fluid of patients sick with the disease, and because the serum of patients contains antibodies against the organism. It has not been encountered in blood cultures in any disease of our experience or to our knowledge and normal human serum does not contain antibodies against it.

We have tentatively named the organism *Haverhillia multiformis*, thereby making a new genus *Haverhillia* in the family *Mycobacteriaceae* (Chester) under the order *Actinomycetales* (Buchanan). Its classification is based on the system which is proposed by the committee on classification headed by Bergey and appointed by the Society of American Bacteriologists. The location of this organism in these divisions is due to its rod shape, usually filamentous or thread form, decided tendency to branching, irregular shapes and swellings, non-motility, requirement of complex proteins for growth and parasitism. Its classification will be discussed later.

Bacteriology

Isolation of *Haverhillia multiformis* was made by putting 5 to 10 cc. of blood from a vein, or joint fluid from a swollen knee into a flask of plain broth (veal infusion containing 0.1 per cent dextrose). At times positive cultures were exposed to two or three hours of freezing temperature in being brought to Boston, indicating resistance to cold. After incubation for twenty-four to seventy-two hours, positive cultures showed darkened blood at the bottom of the flask and on smear numerous short and long gram-negative rods. Subcultures were made in ascitic fluid broth and in clotted rabbit blood.

Identity of Strains. The thirteen strains isolated from the blood and knee joints proved to be identical by microscopic examination, culture reactions in carbohydrate broths, immunity tests with white mice and by cross agglutination and absorption tests with immune rabbit serum.

Morphology. Haverhillia multiformis appears microscopically in various forms, depending largely on the kind of medium and oxygen relations. In general, it is a rod varying in length from 2 or 3 to 10 or 15 microns and in width from 0.2 to 0.5 micron. Many rods have definitely tapering and pointed ends but the majority of the ends are rounded. Associated with other shapes in a field are often seen very short forms, appearing as coccobacilli which are single or in pairs. Often filaments and threads are seen, some extending across the microscopic field. Such filaments may be straight, curved or looped several times. Sometimes they are apparently composed of single cells, but more often they are made up of rods of varying length. Occasional filaments and threads, especially in old cultures, take the stain irregularly, showing successive fine granules in the cell body. Rarely, distinctly wavy filaments are seen. A marked characteristic is the occurrence of swellings, enlargements or knobs. These occur anywhere in the rod — terminally, subterminally or centrally. They vary in size from double to four or five times the diameter of the rod. Their shape is either perfectly round, tapering into the shape of the organism or swelling it to give a fusiform or cigar-shape. Rarely, there is seen a group of two to four such large masses strung together by a fine thread. These enlargements do not take the bacterial spore (Möller) or metachromatic granule (Albert) stains; they stain easily and intensely with the Ziehl-Neelson carbol-fuchsin diluted three times.

There is a tendency to true branching which has been demonstrated by occasional, definite Y-shapes. This branching is seen only when filaments or threads occur and not among the bacillary forms.

The arrangement of the organisms in the microscopic field varies greatly. Rods and short filaments in small groups or clumps lie irregularly, either parallel, crossed or tangled together. In smears of cultures in clotted rabbit blood, they tend to lie parallel with loose rods extending from such a clump or lying free. When filaments and threads predominate, they usually lie in tangled masses, unless vig-

orously streaked out on the slide. In an unstained hanging-drop preparation, the appearance of individual rods, filaments, clumps or masses is not essentially different from that seen in stained preparations.

In experimental local lesions and in blood smears of mice fatally injected, the morphology is regular, the rod shape alone is found and the units lie in large and small clumps without definite arrangement. On the peritoneal surface of rabbits fatally injected intraperitoneally the forms are longer and somewhat tangled. No suggestion of dense colonies with peripheral finger-like projections is found under any cultural or experimental condition.

In general, it can be said that in favorable mediums and under favorable conditions, *Haverhillia multiformis* tends to be regular and rod-shaped, varying in length and occasionally filamentous, and forming compact masses with parallel units. Filaments and threads occur both on solid and in fluid mediums; all varieties of shape may be seen in one preparation. Under unfavorable conditions of oxygen tension, moisture or temperature, the morphology is irregular and bizarre with peculiar knob forms, enlargements, tapering rods and variation in size.

Staining Reactions. Haverhillia multiformis is not easily stained with the ordinary aniline dyes; young cultures are more readily stained than old ones. It is gram-negative and non-acid fast. The enlargements of the bacterium do not take the spore stain. No capsules have been demonstrated and no metachromatic granules are stained by Albert's method.

Colony. The colony in ascitic fluid or animal serum broth is white, soft and not easily broken up. It is 1 to 2 mm. in diameter, round and has the appearance of a miniature cotton-ball. When dislodged from the side of the tube, the mass slowly settles. At the bottom of a tube of fluid medium the growth appears as a grayish white layer that comes up in fine round particles upon agitation of the tube. Because of the tendency to form these clumps, the supernatant fluid is perfectly clear. No pellicle is formed.

Growth in semisolid agar containing ascitic fluid occurs as a diffuse grayish line following the course of inoculation. The colony on the surface of a solid medium is commonly discrete, but if very moist, the growth is somewhat confluent. It is 1 to 2 mm. in diameter, colorless or slightly grayish (as on chocolate agar), and trans-

parent; it is round and regular with a smooth convex surface and very fine radiating lines seen by low-power microscopic examination; it is soft, moist and homogeneous.

Motility. No motility is observed in hanging-drop or dark-field preparations.

Odor. No odor can be detected.

Cultivation. Food Requirements. It was found early in the investigation that *Haverhillia multiformis* grows under artificial conditions with considerable difficulty. No growth occurs on plain agar or in beef infusion broth to which 0.1 per cent dextrose is added. Whole sheep blood agar gives extremely scanty surface growth while on chocolate agar, the colonies are of fair size but not numerous. This organism flourishes, however, in broth to which 5 or 10 per cent defibrinated blood (sheep, rabbit or human) has been added. Fresh whole rabbit blood allowed to clot and inactivated fifteen minutes at 55 C, gives a good medium in which the bacterium grows profusely in twenty-four hours. Broth to which serum (rabbit, sheep, cow, horse or human) is added furnishes heavy growths in twenty-four hours, which when collected give material for study. Ascitic fluid broth is probably the best liquid medium for rapid growth.

The most favorable solid medium that we have found is composed of equal parts of glycerine extract of potato and infusion broth to which egg yolk is added. This is then coagulated in a slanting position in either tubes or bottles. Small amounts of plain broth added to this medium increase its value in detecting the organism in suspected sources, *e. g.*, body fluids of inoculated animals. Loeffler's coagulated serum is not a good medium for this organism. Either potato starch medium, a slice of potato over broth or coagulated egg yolk alone does not support a satisfactory growth. There is a slight growth in broth to which sterile pieces of animal tissue are added. Carbohydrates alone dissolved in broth give scanty if any growth.

The ability of *Haverhillia multiformis* to grow or live in milk was carefully studied because of the epidemiology of the disease. Large and small amounts of inoculum were put into milk treated in various ways and incubated at room temperature and 37 C for various periods of time. A study of Table 1 shows that the results were irregular but in general, milk is distinctly an unfavorable medium either for growth or viability. At room temperature, the organism does not grow and is not recoverable by subculture in a favorable medium.

In repeated tests at 37 C, it was twice seen in smears made by scraping the bottom of the tube with the loop; but ordinarily this was not the case even when such nutriment as whole blood or ascitic fluid was added to the milk. On the other hand, subcultures of a loopful of material from the bottom of tubes of straight milk were positive in some tests up to four days but not at the end of six days incubation. Cultures in milk with sheep blood or ascitic fluid added were positive in subculture but not on smear up to six days. At no time was there any indication of coagulation or acidity, the latter being tested by litmus indicator added either before inoculation or at the end of the test. Controls were always run on the viability of the cultures used in these tests.

Apparently, *Haverhillia multiformis* does not flourish in milk at room temperature or 37 C. It seems not to grow sufficiently to be seen on smear of the sediment of test tubes, but it either grows slightly or persists sufficiently to be subcultured up to four days but not thereafter.

In general, whole blood, defibrinated blood, serum, ascitic fluid, and egg yolk with potato starch seem to furnish the most favorable food constituents. After artificial cultivation for five months, this organism does not appear to be any more easily grown on ordinary laboratory mediums than at first.

Oxygen and Carbon Dioxide Relations. *Haverhillia multiformis* grows on all varieties of blood or fluid mediums in large or small amounts at atmospheric pressure of oxygen. On a solid medium, growth is slow and scanty at atmospheric pressure and slightly better in an anaerobic jar; but the most favorable condition is obtained in a sealed jar in which a candle has been burned. Replacement of all the air with carbon dioxide does not give a better growth than in the candle jar. Slants sealed with paraffin or sealing wax allow a fair but slow growth.

Temperature Relations. These relations were determined by planting fresh cultures in tubes of horse and cow serum broth, on the coagulated egg medium and in sealed tubes of clotted rabbit blood. The tubes were exposed to temperatures ranging from 10 to 55 C. The range of temperature for growth was found to be between room temperature (20 to 22 C) and 40 C. The optimum temperature is between 35 and 38 C. Heating fresh, healthy cultures in clotted rabbit blood kills the organism in fifteen minutes at 55 C. Cultures

TABLE 1

Milk experiments with *Haverhillia multiformis*

Temperature of incubation	Milk	Number of tests	Per cent milk	Medium added	Period of incubation	Results	
						Direct smear	Subculture
20 C.....	fat-free, autoclaved	2	100	....	to 13 days	repeatedly negative	repeatedly negative
	whole, fresh, unsterilized	2	100	....	to 5 days	repeatedly negative	repeatedly negative
37 C.....	fat-free, autoclaved	2	100	....	to 13 days	repeatedly negative	repeatedly negative
	whole, fresh, unsterilized	2	100	....	to 5 days	48 hrs., positive 5 days, negative	not done negative
	whole, autoclaved	2	10	plain broth	to 4 days	positive	not done
	whole, autoclaved	2	33	plain broth	to 4 days	repeatedly negative	not done
	whole, autoclaved	1	90	sheep blood	to 6 days	repeatedly negative	positive
	whole, autoclaved	1	90	ascitic fluid	to 6 days	repeatedly negative	positive
	whole, autoclaved	3	100	....	to 6 days	repeatedly negative	4 days, positive 6 days, negative

of the bacterium in fluid and on solid mediums die in two to four days at 37 C. A twenty-four hour culture in serum or ascitic fluid broth lives a week to ten days in the icebox; in blood broth, about two weeks; in clotted rabbit blood, six weeks; and on the coagulated egg medium, about five to seven days. For carrying the stock cultures, clotted rabbit blood has been constantly used and the transfers have been made weekly.

Reaction of the Medium in Relation to Growth. Growth takes place in broth at pH 7.8 to which blood is added. Straight blood, presumably slightly alkaline, is a favorable medium. Broth to which ascitic fluid is added is pH 8.0 to 8.4 before inoculation and gives an excellent growth.

Moisture Relations. Moisture seems essential to the growth of *Haverhillia multiformis*. This necessitates using either a fresh solid medium, a solid medium to which a small amount of plain broth is added (*e. g.*, the coagulated egg medium), a sealed jar in which a candle is burned or an incubator with atmosphere saturated with moisture.

Reactions in Mediums. This organism causes in blood broths a darkening of the blood with some beta hemolysis that increases with incubation. Whole blood agar plates are slightly changed along the streak of growth. Clotted rabbit blood is also darkened after twenty-four hours incubation. Milk is apparently not changed but growth is so slight that a definite statement regarding the reaction cannot be made.

In order to find what carbohydrates are attacked by this organism, it was necessary to add to the carbohydrate broths a constituent that would encourage growth. Ascitic fluid was used which had a trace of a fermentable sugar in it. Therefore, controls were used to check the reactions. Sugar-free broth was employed. With the Andrade indicator certain reactions were obtained that were confirmed by other tests using suitable indicators. A limited number of carbohydrates has been tested; the findings constitute a preliminary report. The following protocol is an example of the reactions obtained after three days incubation of all tubes. A similar series of tubes showed a partial reaction at twenty-four hours and a complete reaction at seventy-two hours.

TABLE 2

The reaction of *Haverhillia multiformis* on certain carbohydrates

Carbohydrate	Carbohydrate broth with ascitic fluid		Carbohydrate broth without ascitic fluid	
	Inoculated *pH*	Uninoculated *pH*	Inoculated *pH*	Uninoculated *pH*
Dextrose	5.6	7.6	7.0	7.2
Dextrine	5.6	8.0	6.8	7.6
Glycerine	7.8	8.0	7.8	7.8
Lactose	6.6	7.4	7.0	7.4
Levulose	6.4	8.0	7.0	7.4
Maltose	6.6	7.8	7.0	7.2
Mannite	7.8	8.0	7.8	7.8
Saccharose	7.8	8.0	7.8	7.6
Starch	5.0	8.0	5.6	7.8
Control	7.8	8.0	7.8	7.8

All reactions were without the formation of gas. Duplicate tubes incubated seven days gave practically the same results. The table indicates that starch, dextrine and dextrose are vigorously attacked. The reactions with lactose and maltose are relatively weak, and there is a question whether the mild acidity obtained is due to a weak reaction or contamination with small amounts of dextrose split off in sterilizing the medium.

VIRULENCE AND IMMUNITY

The virulence of *Haverhillia multiformis* was studied by tests on white mice, guinea-pigs, rabbits, and a cow and calf.

White mice injected intraperitoneally with twenty-four or forty-eight hour cultures died in sixteen to forty-eight hours. Subcutaneous injections had fatal results but survival was longer. The amounts used varied from 0.5 cc. of a culture in straight sheep blood to the growth from a small slant (tube measuring 12 × 1.3 cm.) of the coagulated egg medium. Nothing distinctive was found in post-mortem examinations. The organism was regularly seen in direct smears and recovered in pure culture from the peritoneal fluid and heart blood. In the former site, the size is always smaller than usual, slender and with pointed ends, while in the heart blood it is thicker, with rounded ends, straight and in small clumps of six to a dozen or more. Feeding a few drops of a concentrated suspension of *Haver-*

hillia multiformis had no effect on two mice. The subcutaneous injection of a pregnant mouse killed in four days without inducing abortion.

Rabbits showed a constant resistance to this organism when injected intravenously, even large doses (*e. g.*, growth from 10 cc. serum broth) causing no symptoms and no localization of infection. Two young rabbits were injected intraperitoneally with massive doses. One died in three and the other in four days with evidences of an acute peritoneal inflammation which in one rabbit extended into the mediastinum. Direct smears of the fibrinous peritoneal exudate and cultures of this exudate in both rabbits and of the heart blood of one showed the injected organism in pure culture. A full-grown rabbit survived a proportionately larger dose injected intraperitoneally. Subcutaneous injections caused no remarkable reaction with small doses, but with a large dose one rabbit had local edema and swelling that subsided in three days leaving only a thickening of the subcutaneous tissues. Intracutaneous inoculations led to an erythematous area 0.3 to 0.5 cm. in diameter with a small yellow necrotic center; about such a lesion the skin was indurated. This reaction appeared in twenty-four to forty-eight hours and faded in two or three days.

One rabbit injected intratracheally survived without symptoms and had a sterile blood culture. Intra-articular injections into both knee joints of two rabbits caused in three to five days acute symptoms of swelling, redness, heat and loss of function; these symptoms subsided in about two weeks. Fluid drawn from such a joint in the acute stage was cloudy and on direct smear showed polymorphonuclear leucocytes, but no bacteria were found either by direct smear or culture. After complete healing, necropsy revealed no abnormality of the joints. Intratesticular injections of several rabbits produced an extreme local reaction of edema of the scrotum and swelling, redness and induration of the testicle with local elevation of temperature. Cultures but not smears of testes removed in the acute stage were usually positive for the organism injected. This reaction appeared in twenty-four hours and in four or five days the acute condition was reduced, leaving a firm nodular testicle.

Two of three rabbits injected intracerebrally died, one in twenty hours and the other in two and a half days. Death followed acute symptoms of nystagmus, lateral turning of the head and rapid,

labored respiration. Necropsy of each rabbit showed a small area of hemorrhage and necrosis at the site of puncture. The organism was seen in one but not in the other in the direct smear from the brain; it was recovered from both brains at the site of injection, and from a distant part. The heart blood yielded a pure culture of the bacterium in one instance but not in the other.

Growth from a twenty-four hour culture of *Haverhillia multiformis* in ascitic fluid broth injected subcutaneously into a pregnant rabbit did not induce abortion. Five cc. of a concentrated suspension of viable organisms administered to a rabbit by mouth had no apparent effect.

Guinea-pigs showed no reaction to intratesticular injections and only a slight local erythema was seen after intracutaneous and subcutaneous injections. Of eight guinea-pigs injected intraperitoneally, two died in three days but their death was complicated by pneumonia which probably was the important element, since heart blood cultures were negative for *Haverhillia multiformis*. One animal showed the injected organism on smear but not on culture of the peritoneal exudate; at no time were any macroscopic or microscopic granules or club forms found in the exudate. Three other guinea-pigs were found dead after ten days, two months and three months, but postmortem and culture examinations were negative and they may be considered to have died of some intercurrent disease. Feeding 2 cc. of a concentrated suspension of organisms by means of a catheter had no effect.

A *cow* and her suckling *calf* were procured to attempt to reproduce the epidemiology of the disease. We had found that patients showed a high titer of antibodies and we had demonstrated antibodies in the serum of one cow on the suspected farms. If the cow is susceptible and if we could give the experimental animal an intravenous inoculation of such a strength that it would produce an infection either generally or in the udder, she might excrete the organism in her milk. This would then be taken by the calf which also might react with the production of antibodies. If these conditions of the epidemic could be repeated, it would furnish strong evidence in support of the epidemiology.

In preliminary tests we found that the cow and calf had no antibodies against *Haverhillia multiformis* and that the organism could not be demonstrated in the milk by culture or smear of sediment.

TABLE 3

Summary of animal experiments

Animal	Number injected	Number of strains used	Route	Number recovered	Number died	Average period of survival	Findings
White mice..	22	5	Intraperitoneal	4	18	29 hours *	Injected organism in heart blood culture of 16 mice
	3		Subcutaneous	0	3	5 days	Injected organism in heart blood culture of 3 mice
	2	1	By mouth	2	0	....	No effect
Guinea-pigs .	8	5	Intraperitoneal	3	2	3 days	Peritonitis complicated with pneumonia; heart blood cultures negative
					3	10 days 2 months 3 months	Postmortem examinations and cultures negative
	2	2	Intratesticular	2	0	....	Negative
	1	1	Intracutaneous	1	0	....	Very slight erythema
	1	1	Subcutaneous	1	0	....	Slight edema and thickening
	1	1	By mouth	1	0	....	No effect
Rabbits.....	8	4	Intravenous	8	0	....	No reaction
	3	2	Intraperitoneal	1	2	3 days	Acute peritonitis and positive heart blood cultures
						4 days	Acute peritonitis; negative heart blood cultures
	1	1	Subcutaneous	1	0	....	Temporary edema and induration of subcutaneous tissues
	4	1	Intracutaneous	4	0	....	Small area of erythema with necrotic center
	6	1	Intratesticular	6	0	....	All signs of acute local inflammation; local cultures positive
	1	1	Intratracheal	1	0	....	No reaction
	2	2	Intra-articular	2	0	....	Acute local inflammation
	3	2	Intracerebral	1	2	2½ days	Local reaction; cultures positive
	1	1	By mouth	1	0	....	No effect
Cow.....	1	1	Intravenous	1	0	....	Febrile reaction; no antibodies
			Subcutaneous (3 injections)			....	Temporary local swelling and tenderness followed by induration; complete and rapid healing
Calf.....	1	1	Suckling	1	0	....	Taking milk from mother did not produce antibodies
			Subcutaneous (3 injections)			....	No effect

* This figure excludes one mouse that died after 5 days and whose heart blood culture showed a member of the *Bact. coli* group but not the organism injected.

We injected the cow in the jugular vein with the growth from a forty-eight hour culture in eleven 100 cc. flasks of serum broth. She had a temporary reaction, but was eating and appeared normal before the end of the day. Her rectal temperature began to rise the second day after injection, reaching 103.5 F on the third day; the temperature continued to vary from 100 to 104 F, gradually subsiding in three weeks. Blood cultures were persistently negative except for one positive culture taken the day after inoculation. Repeated milk examinations were negative. Agglutination tests of the serum from cow and calf were negative ten and twenty days after the inoculation of the cow.

In order to test further the susceptibility or resistance of the cow and calf to *Haverhillia multiformis* we injected them subcutaneously each three times with large concentrated suspensions of this organism. The calf did not react at all and in two or three days the sites of inoculation were entirely normal. The cow showed tender, swollen, subcutaneous masses at the sites of injection, but these became small and indurated in five days and in twelve days had disappeared entirely.

We were not able to set up in the cow, by the single large intravenous injection, an infection capable of producing antibodies or to infect the calf through the milk. Repeated subcutaneous injections of large doses were innocuous to both animals.

Increase and Decrease in Virulence. No increase in virulence was noted after passage through an animal. After artificial cultivation for five months, as small a dose as at the beginning of the investigation was fatal for white mice. Likewise, intracerebral and intratesticular injections into rabbits produced the same lesions in the same time as in the earlier experiments.

Immunity. Four mice recovered from sublethal and dead cultures. One such injection rendered them immune to doses of homologous and heterologous strains of *Haverhillia multiformis* fatal to control mice. This immunity was found to persist at least two months. Table 4 is a summary of these immunity experiments with white mice. Feeding did not confer protection against subsequent injections lethal for control mice.

TABLE 4

Immunity experiments with white mice

	Same as immunizing strain	Different from immunizing strain					
IMMUNE MICE						CONTROL MICE	
Mouse number	I_2	I_1	I_2	I_3	I_4	Number injected..	9
Times injected.....	3	1	2	6	6	Number died.....	9
Times recovered	3	1	2	6	6	Positive heart blood cultures	8

SEROLOGY

EXPERIMENTAL TESTS

Rabbits injected intravenously four to six times with increasing doses of viable organisms produced immune serums that gave positive precipitin reactions. Antigens for this purpose were prepared in two ways: (1) by growing a strain on the coagulated egg medium, incubating the growth washed off in 0.5 per cent phenol for twenty-four hours and clearing by centrifuging; and (2) by collecting the supernatant fluid of old ascitic fluid broth cultures of all strains. Both antigens diluted 1:20 gave positive precipitin reactions with immune rabbit serum and not with normal rabbit serum.

The serum of rabbits similarly immunized repeatedly agglutinated *Haverhillia multiformis* in dilutions reaching 1:5000 while normal rabbit serum did not agglutinate the same suspension above 1:20. Various antigens were used, the best being the growth from a twenty-four hour culture in ascitic fluid or serum broth resuspended in normal salt solution. Other antigens tried were fresh and old cultures from the coagulated egg medium, which were used either untreated or after repeated freezing and thawing; such antigens, however, were not so satisfactory as when prepared from the fresh cultures described. It was found that shaking the mixture of immune serum and suspension for ten minutes before incubating hastened but did not alter the reaction. This was adopted as a routine measure in all agglutination tests. Incubation at 37 C in a water bath for one hour is sufficient, but at the end of two hours the reaction is more complete. There is some danger of spontaneous clumping after two or three hours incubation so that a close observation of the salt solution and normal serum controls is necessary.

The serum of a rabbit immunized against only one of the strains reacted similarly with suspensions of all other strains (Table 5).

Absorption of antibodies from this serum both by the homologous strain and by a heterologous strain removed all agglutinins against the other strains as well (Table 6). These tests indicate further the identity of the strains recovered from the patients.

TABLE 5

Cross agglutination tests with serum of rabbit immunized against one strain

Strain	Serum of R 1–154				Control in salt solution	Serum of normal rabbit	
	1 : 100	1 : 200	1 : 500	1 : 1000		1 : 50	1 : 100
No. 150.....	3	3	2	1	0	0	0
No. 152.....	4	3	3	2	0	0	0
No. 154.....	4	4	3	2	0	0	0
No. 162.....	4	4	3	2	0	0	0
No. 187.....	4	3	3	2	0	0	0
No. 212.....	4	4	3	1	0	0	0
No. 213.....	4	3	2	1	0	0	0
No. 214.....	4	4	2	1	0	0	0
No. 256.....	4	4	3	2	0	0	0
No. 258.....	4	4	3	2	0	0	0
No. 259.....	4	4	4	2	0	0	0

Legend: Figures refer to degree of agglutination reaction; 4 = complete reaction.

TABLE 6

Absorption tests with serum of rabbit immunized against one strain

Serum of R 1–154 absorbed with strain No. 154

Strain	Absorbed serum 1 : 100	Unabsorbed serum 1: 100	Strain	Absorbed serum 1 : 100	Unabsorbed serum 1 : 100
No. 150....	0	4	No. 212....	0	4
No. 151....	0	4	No. 213....	0	4
No. 152....	0	4	No. 214....	0	4
No. 154....	0	4	No. 256....	0	4
No. 187....	0	not run	No. 258....	0	not run

Serum of R 1–154 absorbed with strain No. 256

No. 150....	0	No. 212....	0
No. 151....	0	No. 213....	0
No. 152....	0	No. 214....	0
No. 154....	0	No. 256....	0

Legend: See Table 5.

Complement fixation tests were positive with an immune rabbit serum and not with a normal rabbit serum. The antigens used were the supernatant fluids of carbolized growths from the coagulated

egg medium and of the growth in old ascitic fluid broth cultures, repeatedly frozen and thawed.

As stated before, we were unable to demonstrate agglutinins in the serum of the experimental cow injected intravenously with a large single dose of the bacterium. The calf which fed only from its mother likewise showed no agglutinins in repeated tests.

TABLE 7

Agglutination tests of patients' serum with polyvalent antigen

	Serum dilution				
Patient's serum	1:20	1:50	1:100	1:200	1:500
No. 150	4	4	4	3	0
No. 151	4	4	4	4	1
No. 152	3	2	1	0	0
No. 153	4	3	2	1	0
No. 164	4	3	2	1	0
No. 212	4	3	2	1	0
No. 213	4	4	3	1	0
No. 218	4	4	4	1	0
No. 256	4	3	2	1	0
No. 258	4	3	2	1	0

	Serum dilution			
Immune rabbit serum control	1:100	1:500	1:1000	1:5000
R 3-214	4	4	3	2

Legend: See Table 5.

CLINICAL TESTS

The serum of *patients* sick with the disease was collected at the time of the epidemic but difficulty in growing the organism recovered from them and in preparing a suitable antigen necessitated delaying the serologic tests for three months. At that time, agglutination tests were carried out with positive results on the serum of ten patients of which nine had given positive and one negative blood cultures. Two samples were received from some patients in different stages of the disease. The serum of two patients with positive blood cultures was not available. The ten serums on hand reacted similarly in three sets of experiments with polyvalent antigens prepared as for the rabbit tests above. The titers of the various serums ranged between 1:20 and 1:500 with the majority of the titers at 1:50 and 1:100. These results are given in Table 7. Control tests were made at the same time on the serum of forty normal persons. These were consistently negative in dilutions above 1:20 and only two gave weak reactions in that dilution.

The blood of the *cows* whose milk was being sold at the time of the epidemic was obtained three months later. These included the cows of the three farms and numbered fifteen. Cultivation of all specimens was negative bacteriologically for *Haverhillia multiformis*. The serums from these specimens were tested for agglutinin content in an effort to trace the source of the organism with which the patients' serum reacted. Three series of tests were run with various antigens prepared as for the tests with rabbit serum. As controls, the serum of nine cows taken at random at the local abattoir was used. The serum of one cow (cow 11) on the farm of the milk dealer agglutinated *Haverhillia multiformis* completely in the dilution of 1: 100 and partially in 1: 200. Of the other cows on the three farms and the nine control cows, the serum of a few gave a partial reaction in a dilution of 1: 20 but not above.

Nothing remarkable was noted regarding the cow that reacted positively except for a superficial lesion on one teat and another teat that dripped milk constantly. The local and state veterinarians inspected the herds on the three farms and pronounced them apparently well. Unfortunately, this cow could not be studied thoroughly because of the distance of the farm from Boston and because of the hostility of the farmer; when steps were taken to purchase the cow for study, it was learned that she had been sold to the butcher.

Microscopic Pathology

R 1–151, a young rabbit, was injected intraperitoneally with a large dose of *Haverhillia multiformis* and died three days later with gross evidence of acute fibrinous peritonitis with extension into the mediastinum and pleural cavities. A brief account of the microscopic pathology follows. *Heart:* Negative. *Lung:* Necrotic cellular débris and clumps of the organism are on the pleural surface. *Spleen:* Polymorphonuclear and endothelial leucocytes with necrotic cellular débris are found on the surface with masses of the bacterium injected seen as intertwining threads; otherwise the spleen is negative. *Liver:* Large numbers of the organism with fibrin and polymorphonuclear leucocytes are on the surfaces; there are foci of necrosis of the liver cells near the capsule; elsewhere the liver is negative. *Gastro-intestinal tract:* Many polymorphonuclear leucocytes, fibrin and masses of bacteria are on the peritoneal surface; otherwise the tract is negative. *Adrenals* and *kidneys:* Nega-

tive. *Bone marrow:* Extensive necrosis of hematopoietic cells including the megalokaryocytes is found; many of these latter cells are apparently degenerated and contain pink-staining hyaline masses in their cytoplasm.

A testicle from each of two rabbits inoculated intratesticularly was removed after forty-eight hours (R 2–256) and seven days (R7–256). Microscopically the forty-eight hour specimen shows a more acute condition, namely, an extensive necrosis of the germinal epithelium and connective tissue with fibrin in places in the necrotic material. The periphery of this necrotic area is infiltrated with polymorphonuclear leucocytes; about the lesion are many such leucocytes together with endothelial leucocytes of which large numbers contain cellular débris. The typical bacteria occur in clumps as short to medium length rods.

The other testicle (R 7–256) removed seven days after inoculation shows an area of necrotic germinal epithelium and connective tissue with fibrin and edema surrounded by an area of necrotic tissue infiltrated with polymorphonuclear leucocytes many of which are dead. This whole area is becoming encapsulated by granulation tissue showing some fibroblasts growing into the necrotic tissue; in this granulation tissue, endothelial leucocytes and lymphocytes are seen. No bacteria are found anywhere in the lesion. *Heart, spleen, liver* and *kidneys* of this rabbit are negative.

Two rabbits died following intracerebral inoculations. R 5–256 died after twenty hours and shows microscopically at the site of the inoculation a small area of necrosis in the cortex which is infiltrated with polymorphonuclear leucocytes. About the lesion are focal hemorrhages. In the necrotic tissue and extending into the surrounding area are found masses of short and medium length bacteria without irregularity of shape. Elsewhere the brain is normal.

R 4–256 died seventy hours following inoculation and microscopic examination of the organs follows. *Brain:* There is an area of necrosis in the cortex with hemorrhage and infiltration with polymorphonuclear leucocytes. Scattered around this area are small focal hemorrhages and in the necrotic tissue are seen clumps of short and medium length bacillary rods. The meninges in the vicinity of the cortical lesion show polymorphonuclear leucocytes, fibrin and dilated blood vessels. Elsewhere the brain is normal. *Liver:* Sections of this organ show focal necrosis and hydrops of cells in the center of the lobules. *Spleen* and *kidneys* are negative.

Discussion

The identification of this organism isolated from the blood and knee joint fluid of patients is a matter of some difficulty. Such a pathogenic bacterium has not been met by us and we have found no reference to it in books or literature on systematic bacteriology. The disease with which this organism was associated is unusual and has been given a distinctive name. These facts lead us to believe that it is a hitherto undescribed parasitic microörganism.

In classifying this organism, we followed the system as presented by the committee on classification appointed by the Society of American Bacteriologists.[2] It belongs, we believe, among the actinomycetes as representing that large group of microörganisms whose cells are elongated and frequently filamentous with decided tendency to branching. Swellings, clubs and irregular shapes of the cells and the lack of motility and endospores are other features common to our organism and this group of higher bacteria. We eliminated the possibility of our bacterium being *Actinomyces bovis* because it is gram-negative and forms neither granules, star-shaped masses with finger-like projections nor marked branching. The actinobacillus of Lignières and Spitz[3] received considerable consideration because of the epidemiology of Haverhill fever and the general similarity of that bacillus and our organism. But we concluded that they are different because the actinobacillus is pathogenic for guinea-pigs with the formation of granules in the pus after intraperitoneal injection, and because of the presence of radiating masses in the pus, the facility of growth in ordinary laboratory mediums including milk and the surface of plain agar, turbidity in broth with formation of a pellicle, the necessity of body temperature for growth, the adherence of the colony to solid mediums, the arrangement as a coccobacillus or diplococcus and finally the characteristic of bipolar staining. *Haverhillia multiformis* has none of these characteristics.

We believe our organism falls in the family *Mycobacteriaceae* (Chester) because of its parasitism, rod shape with frequent irregularity in form, slight or occasional branching and occasional uneven staining. The genera in this family as outlined in Bergey's Manual of Determinative Bacteriology do not include our organism because of acid-fast or gram-positive staining, obligate parasitism and inability to ferment carbohydrates. It approaches genus IV the most

closely but is distinct from it since the genus *Pfeiferella* does not attack carbohydrates and our organism does. Consequently, we propose the formation of a new genus (Genus V) with the name of *Haverhillia* and the type species as *Haverhillia multiformis*. The name is based on the town in which the disease was first studied and the most out-standing characteristic of the organism. A description of the type species follows:

> Slender, gram-negative and non-acid resisting rods staining with some difficulty; often forming threads and showing tendency toward branching; marked irregularity of form with swellings and enlargements; fermentation of some carbohydrates; in general, requiring blood or ascitic fluid for growth.

The relation of *Haverhillia multiformis* to the disease as the specific etiologic agent is based on its recovery from the blood and joint fluid from twelve of twenty-two patients suffering with the disease and on the finding of specific agglutinins for the organism. The bacterium is pathogenic for white mice and rabbits and has the characteristics of parasitism in being difficult to grow under artificial conditions and in preferring body temperature. Numerous controls on the medium in which the cultures were taken, syringes used and technic of taking the cultures prevented any possibility of this being a contaminating organism. Because of these facts, we believe that we isolated the specific etiologic agent of Haverhill fever and that it is a pathogen and not a contaminating saprophyte.

We have incomplete data on the source of the organism. Epidemiologically, the disease was traced to milk. We found, on the other hand, that under artificial conditions *Haverhillia multiformis* does not flourish in milk. We found it to live in milk for four days at body temperature but the increase was slight, if any. It is conceivable that large numbers in the milk can survive and reach humans under natural conditions. Presuming that this might be possible, we looked among the incriminated herds for sources of the organism. Milk cultures taken at the time of the epidemic were negative, but such tests are not entirely reliable. Because of certain difficulties, blood cultures of the cows were, unfortunately, not made until three months after the epidemic; at that time they were negative. Our only evidence supporting the belief that the cow is the source of the organism is the fact that the agglutinin titer of one cow was 1: 100.

The tests of the serum of the other cows on the three farms and several unsuspected cows were at most only slight at the 1: 20 dilution at which a reaction is negligible. The evidence involving this one cow is not to be overlooked; however, evidence must yet be presented to show that one cow could scatter sufficient organisms through a medium experimentally unfavorable for the organism and diluted with other cows' milk to produce such a widespread epidemic. Furthermore, in a necessarily limited number of experiments, we found a cow not susceptible to this organism. This problem is more suited for experimentation on a larger scale under proper facilities.

We have no direct evidence as to the mode of entry of *Haverhillia multiformis* into the human body. Human experiments were not done. Feeding animals experimentally was unsuccessful. Injection of the organism was the only means found to infect experimental animals.

The experimental pathology of *Haverhillia multiformis* shows that lesions having the characteristics of acute inflammation are produced by local injections which if not fatal go on to healing without the production of chronic conditions or abscesses. Nothing like actinomycosis or actinobacillosis is found grossly or microscopically. Polymorphonuclear leucocytes are called out in the acute stages, followed in repair by endothelial leucocytes, lymphocytes and fibroblasts. The only peculiar lesion that has been found is the change in the bone marrow in which the megalokaryocytes show necrosis with pink-staining hyaline masses in the cytoplasm.

Summary and Conclusions

A report of the bacteriology, serology and experimental pathology of a bacterium isolated from patients suffering with Haverhill fever is given. Evidence is presented to show that this organism is the specific etiologic agent of the disease. The importance of taking blood cultures in similar conditions is emphasized, as such a procedure proved of great value in this investigation.

The organism seems to belong to the order *Actinomycetales* (Buchanan) and in the family *Mycobacteriaceae* (Chester). Since it has characteristics incompatible with the genera of this family, a new genus is proposed and the organism has been tentatively named *Haverhillia multiformis*.

No direct evidence of the source of the specific bacterium in the epidemic has been found, although the serum of a cow in the suspected herds contained agglutinins against the organism. Experimental data to explain the mode of entry into the human body were not obtained.

Credit is due Miss Marion E. Lamb of this laboratory for first demonstrating this bacterium in the blood cultures.

We are extremely grateful to Dr. F. B. Mallory for taking the photomicrographs. A note on the technic follows.

Wellington ortho process contrast plates were employed in making all negatives. The photomicrographs were taken with a Zeiss outfit supplied with a tungsarc light. A 2 mm. apochromatic oil immersion objective and a No. III homal ocular were used with a Wratten and Wainwright color screen B. The organisms were stained deeply with gentian violet.

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2. Bergey, D. H., Harrison, F. C., Breed, R. S., Hammer, B. W., and Huntoon, F. M. Manual of Determinative Bacteriology. Baltimore, 1923.
3. Lignières, J., and Spitz, G. Contribution to the study, classification and nomenclature of the affections known under the name of actinomycosis. *Centrbl. f. Bakteriol.*, Abt. 1, Orig. 1904, xxxv, 452.

DESCRIPTION OF PLATES

PLATE 71

FIG. 1. Twenty-four hour culture in ascitic fluid broth incubated and photographed in slanting position. × 2.

FIG. 2. Forty-eight hour culture in ascitic fluid broth. Contents of flask poured carefully into flat-bottomed glass dish and photographed. × 2.

PLATE 72

FIG. 3. Twenty-four hour culture in clotted rabbit blood. Note regularity of shape and parallel arrangement. × 2000.

FIG. 4. Twenty-four hour culture on the coagulated egg medium; incubated in sealed jar in which candle was burned. Note frequent streptobacillary and coccobacillary forms. × 2000.

PLATE 73

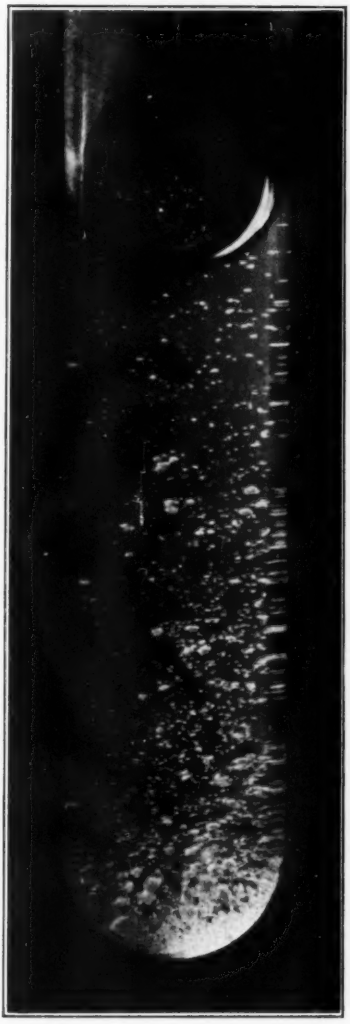
FIG. 5. Another preparation of the same culture and incubation as Fig. 4; showing more irregularity of shape and size with occasional fusiform and swollen rods. × 2000.

FIG. 6. Eight day culture on the coagulated egg medium; incubated in sealed jar in which candle was burned. A tangle of threads and filaments showing occasional thick forms and Y-shapes suggesting branching. × 2000.

PLATE 74

FIG. 7. Another field of the same preparation as Fig. 6. Irregular forms and large swellings in threads and filaments; occasional branching form. × 2000.

FIG. 8. Another preparation of the same culture and incubation as Fig. 6. Marked irregularity of shape and size with some filaments staining irregularly. × 2000.



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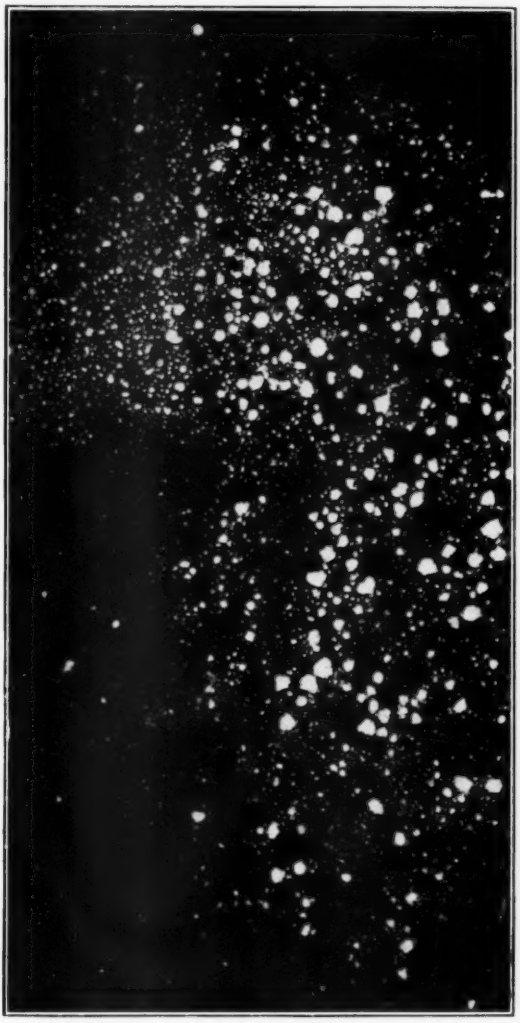

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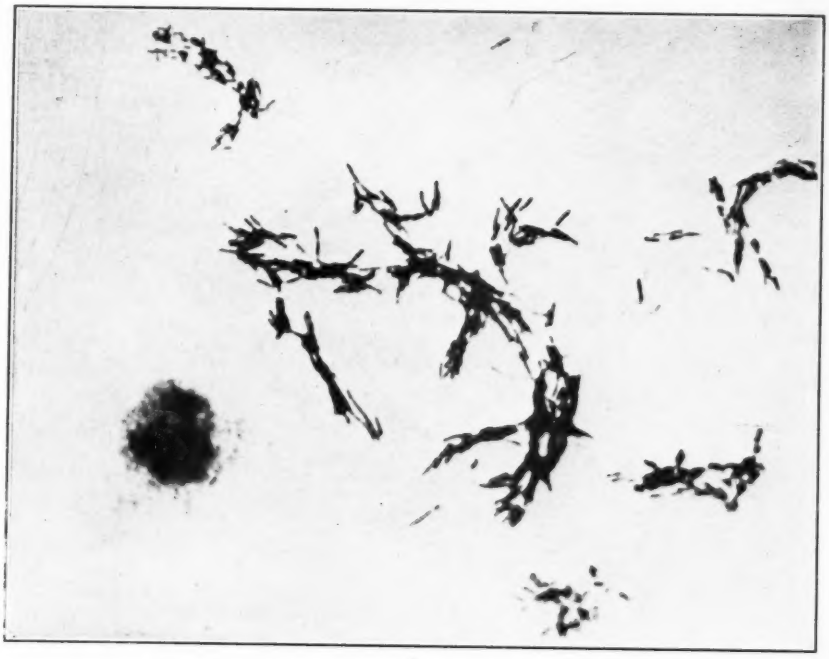

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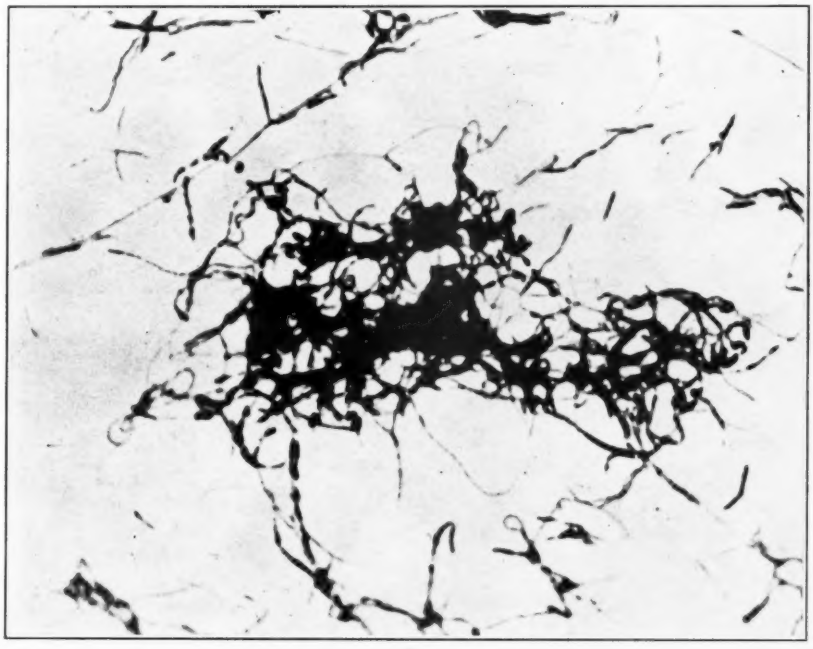

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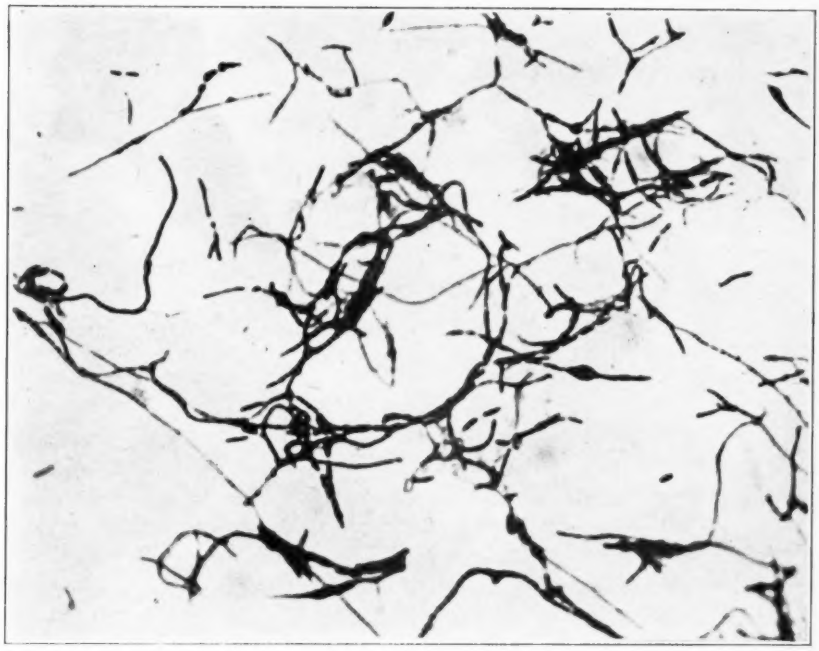

7

8

SO-CALLED "ENDOTHELIAL BLOCKADE" WITH COLLARGOL

AN IMMUNOLOGIC AND HISTOLOGIC STUDY *

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Much has been written in recent years on the results produced by the injection of various colloidal suspensions, with special attention devoted to the effect on the so-called reticulo-endothelial system. Jungeblut and Berlot [1] in one of their recent publications give a good review of the literature, a summary of which would show somewhat conflicting reports as to the effect of so-called "endothelial blockade." These two authors, utilizing injections of India ink, delayed antitoxin formation in guinea-pigs; [1] in a second paper,[2] they claimed to have shown diminution in complement following similar injections. Gay and Clark,[3] employing trypan blue, demonstrated diminished antibody production in rabbits, while Lewis and Loomis [4] found the converse to be true; this discrepancy the latter authors reconciled on the basis of the general physiologic law that substances which are stimulating in certain doses are depressing in larger amounts. Isaacs,[5] working with trypan blue, apparently found diminished hemolysin production in guinea-pigs but no protection against anaphylactic shock. Howell and Tower [6] found that nine to nineteen injections of 2 to 6 cc. each of saccharated iron oxide did not influence typhoid agglutinin titers in rabbits. Some of these workers use the term "endothelial blockade" without justification in our opinion, as they failed to control their results histologically; this is especially true as regards trypan blue for this dye is easily diffusible and is found in epithelium in as large if not larger amounts than in endothelium. It likewise persists in considerable amounts for a long time in the plasma.

We decided to attempt work along these lines, using for our injections collargol, a substance that is difficultly diffusible, that is

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taken up practically exclusively by endothelium and allied cells and that is not easily eliminated. We also planned a careful histologic study in order to determine the location and amount of collargol taken up by the tissues and also to see if any correlation between the morphologic and immunologic findings was possible. The collargol used in these experiments was that manufactured by the Heyden Chemical Works and distributed by Shering and Glatz, New York. It was used either in 1 per cent or 2 per cent suspension in sterile distilled water and filtered immediately before use. We used both guinea-pigs and rabbits.

Experiments with Guinea-pigs

We first attempted to inject our guinea-pigs intracardially with collargol but had to discontinue this method since such injections proved fatal, apparently due to the fact that collargol caused the formation of intracardiac clots. We then used the ear veins and, if necessary, the leg veins and jugulars. We found the largest dose which could be safely employed to be 1 cc. of a 1 per cent suspension. As antigen, we used in our first and second series hen blood, for we hoped to be able to study phagocytosis of the nucleated erythrocytes; this however proved unsatisfactory and so in our third series we used sheep blood.

The first series consisted of five guinea-pigs, three serving as controls. The collargol guinea-pigs were injected as follows.

Guinea-pig 1 received a total of six injections of 1 cc. each, in a period of nine days. On the thirteenth day after the first injection, it received 3 cc. of 16 per cent washed and packed hen cells subcutaneously and a like amount intraperitoneally. Its serum was titrated on the ninth and twenty-second day following this injection. The animal was killed at the time of the last bleeding and its organs preserved for study.

Guinea-pig 2 was given a total of four injections of 1 cc. each in a period of five days. On the sixth day it was given 4 cc. of 16 per cent washed and packed hen cells intravenously. It was found dead the next morning showing grossly an enlarged spleen and fatty liver. The histologic picture will be described below.

Two other guinea-pigs received 2 cc. of 16 per cent washed and packed hen cells intravenously and were killed in four and twenty-seven hours respectively for histologic purposes.

A control guinea-pig received the same amount of hen cells as Guinea-pig 1 and its serum was titrated at the same time intervals.

The results of the titrations of these serums at the twenty-second day bleeding are given in Tables 1 and 2, Table 1 giving the agglutinin and Table 2 the hemolysin titers.

TABLE 1

Antigen: 2 per cent hen blood.
Incubated 2 hours at 37.5 C, then overnight in icebox.

Serum Dilutions	Guinea-pig 1	Control
1:10	++++	++++
1:20	++++	++++
1:40	+++	++++
1:80	–	++++
1:160	–	+++
1:320	–	–

TABLE 2

Antigen: 2 per cent washed and packed hen cells, 0.25 cc.
Complement: 0.3 cc. 1:10 guinea-pig serum.
Total volume: 1.25 cc.
Incubated 1 hour at 37.5 C.

Serum	Guinea-pig 1	Control
0.05	C	C
0.025	C	C
0.01	o	C
0.005	o	C
0.0025	o	o
0.0001	o	o

(C = complete hemolysis; P = partial hemolysis;
o = no hemolysis)

The second series consisted of five collargol guinea-pigs and two controls. The injections each of 1 cc. are given below:

Guinea-pig 3 — six injections in the course of ten days
" " 4 — five injections in the course of nine days
" " 5 — six injections in the course of eleven days
" " 6 — four injections in the course of four days
" " 7 — five injections in the course of five days

All animals received in the jugular vein 3 cc. of defibrinated hen blood, diluted 1:3, four to six days following their last collargol injection. Guinea-pigs 3 and 6 were killed six hours later for histologic

study. The others, including two normal animals that were similarly injected with hen blood, were bled from the heart on the ninth and twenty-second day and their serums titrated. Guinea-pig 5 was killed on the twenty-fifth day for histologic purposes. On the twenty-sixth day Guinea-pigs 4, 7 and the two controls were given in the jugular veins 3 cc. of defibrinated hen blood, diluted 1:3. The results are given below, Table 3 showing the agglutinin titer, Table 4 the precipitin titer, both on the twenty-second day, and Table 5 the shock experiments on the twenty-sixth day.

TABLE 3

Antigen: 1 per cent washed and packed hen cells.
Incubated: 2 hours at 37.5 C, overnight in icebox.

Serum Dilutions	Guinea-pig 4	Guinea-pig 7	Control 1	Control 2
1:10	++++	++++	+++	+++
1:20	++++	++++	–	–
1:40	++++	++++	–	–
1:80	++++	+++	–	–
1:160	+++	–	–	–

TABLE 4

Antigen: varying dilutions hen serum.

Antigen Dilutions	Guinea-pig 4	Guinea-pig 7	Control 1	Control 2
1:2	+	–	*not done*	*not done*
1:5	±	–	*not done*	*not done*
1:10	–	–	++	++
1:20	*not done*	*not done*	++	++
1:50	–	–	++	++
1:100	–	–	+	+
1:500	*not done*	*not done*	–	–

TABLE 5

Injected in jugular vein with 3 cc. hen blood, diluted 1: 5.

Guinea-pig	Result	Remarks
4	Survived	Showed anaphylactic symptoms immediately after injection but recovered.
7	Survived	Same as above.
Control 1	Dead in 4 minutes	Typical anaphylactic symptoms and necropsy findings.
Control 2	Dead in 3¼ minutes	Same as Control 1.

The collargol guinea-pigs show about eight to sixteen times the agglutinin content of the controls, whereas the converse is true as regards the precipitins where the collargol animals show one-twentieth to one-hundredth as great a titer. In the shock experiments, the results are very clean out; the two controls died of typical shock in three and one-fourth and four minutes respectively whereas the collargol guinea-pigs survived.

The third series was made up of five collargol guinea-pigs and two controls. Two of the collargol guinea-pigs unfortunately died before the completion of the experiment. They were injected as follows.

Guinea-pig 8 — 6 cc. collargol in seven days, then 3 cc. defibrinated sheep blood, diluted 1:3, intravenously, followed by 3 cc. more of collargol in four days.
Guinea-pig 9 — 5 cc. collargol in seven days, then 3 cc. defibrinated sheep blood intravenously, diluted 1:3, followed by 3 cc. more of collargol in six days. This guinea-pig died following the last collargol injection.
Guinea-pig 10 — 3 cc. collargol in three days; death after last injection.
Guinea-pig 11 — 6 cc. collargol in seven days, sheep blood as Guinea-pig 8, then 3 cc. collargol in four days.
Guinea-pig 12 — 4 cc. collargol in seven days, sheep blood as Guinea-pig 8 then 1.6 cc. collargol in four days.
Controls 1 and 2 — 3 cc. defibrinated sheep blood, diluted 1:3.

The collargol survivors and the controls were bled on the fourteenth and twenty-first day following the injections with sheep blood and their serums titrated for antibodies. On the twenty-second day, they were tested for sensitization. Table 6 gives the agglutinin titers on the fourteenth day, Table 7 the same on the twenty-first day, Table 8 the precipitin titers on the twenty-first day and Table 9 the shock experiments on the twenty-second day.

TABLE 6

Antigen: 1 per cent washed and packed sheep cells.
Incubated: 2 hours at 37.5 C, overnight in icebox.

Serum Dilution	Guinea-pig 12	Guinea-pig 8	Guinea-pig 11	Control 1	Control 2
1:4	+++	++	+	++++	++++
1:10	+++	+	−	++++	+++
1:20	++	−	−	+++	++
1:40	±	−	−	+++	+
1:80	−	−	−	±	±
1:160	−	−	−	−	−

TABLE 7

Antigen: 1 per cent washed and packed sheep cells.
Incubated: 2 hours at 37.5 C overnight in icebox.

Serum Dilutions	Guinea-pig 12	Guinea-pig 8	Control 1	Control 2
1:4	++++	+++	*not done*	*not done*
1:10	++++	+++	++++	++++
1:20	++++	++	++++	++++
1:40	+++	−	++++	+++
1:80	++	−	++++	+++
1:160	−	−	+++	++
1:320	−	−	+++	−

TABLE 8

Antigen: varying dilutions sheep serum.

Antigen Dilutions	Guinea-pig 12	Guinea-pig 9	Control 1	Control 2
1:2	+	−	−	+
1:10	−	−	++	−
1:50	−	−	+	−

TABLE 9

Injected in jugular veins with 3 cc. sheep blood, diluted 1: 3.

Guinea-pig	Result	Remarks
12	Died in 45 minutes	Typical anaphylactic symptoms following injection, with respiratory distress until death.
8	Survived	Very mild symptoms.
Control 1	Dead in 54 minutes	Typical anaphylactic symptoms following injection, with respiratory distress until death.
Control 2	Dead in 4½ minutes	Typical anaphylactic death.

In this series, the collargol guinea-pigs showed a lower agglutinin titer than the controls at nine days; at this time none showed demonstrable precipitins. On the twenty-first day, the collargol guinea-pigs again were lower in their agglutinin content. Guinea-pig 12, which received a total of 5.6 cc. collargol, shows less difference from the controls than Guinea-pig 8 which received a total of 9 cc. As regards precipitins, Control 1 shows more than either collargol guinea-pig, whereas the titer of Control 2 is about the same as Guinea-pig 12 but greater than Guinea-pig 8. In the shock experiments, the two controls died, Control 1 in fifty-four minutes and Control 2 in four and one-half minutes (the slow death of Control 1 may have been due to its high content of precipitins). Guinea-pig 8 survived. Guinea-pig 12 died in forty-five minutes; the death of this

animal was not surprising for, as mentioned above, it had received considerably less collargol than Guinea-pig 8 and its anti-body titer throughout had more nearly approached the controls.

Discussion

We realize that our number of animals is small; its size, however, was due to the unavoidable difficulties of repeatedly injecting guinea-pigs intravenously with a substance such as collargol for if any of it escapes into the surrounding tissues, the veins in that region are useless for reinjection. Also the time required for the injections was great and unfortunately we had but a limited amount at our disposal. However, the results taken as a whole are sufficiently suggestive to be of some value.

Summarizing then, two facts stand out: the effect of collargol on circulating antibody formation and on anaphylactic shock. We will discuss these two facts in greater detail. The precipitin titer was determined in the second and third series and in both these series it was definitely lower in the collargol-injected guinea-pigs than in the controls. The agglutinins on the other hand showed no such relation, for in the first and third series they were lower in the collargol guinea-pigs than in the controls, while in the second series they were higher. Thus it appears that while collargol may either depress or stimulate agglutinin production, it depresses precipitin formation. Such facts suggest the thought that perhaps different cells or mechanisms are involved in the formation of antibodies against soluble antigens, such as serums, and against particulate antigens, such as blood cells. However, the question is extremely complicated and requires much further study.

The second point under discussion, the effect of collargol on anaphylactic shock, is interesting but likewise is difficult to explain. Three of the four collargol guinea-pigs survived, the animal which died being the one that received the least collargol. The explanation of this apparent protection is very complicated and cannot be simply "endothelial blockade," because the effect of repeated injections of such a substance as collargol means repeated injections of a substance primarily toxic in itself and furthermore of a foreign protein, egg albumen, and a heavy metal, silver; any or all of these factors may be the primary cause and obviously no deduction is justified at the present time.

Experiments with Rabbits

Seven rabbits were used in the first series of experiments — five were injected with collargol and two were used as controls. The five were injected as follows:

They were given intravenous injections daily of collargol, the first four injections of 1 per cent, thereafter of 2 per cent. After they had received an amount corresponding to 22 cc. of 1 per cent collargol, they were given intravenously three injections on alternate days of defibrinated hen blood, diluted 1:3 (1 cc., 2 cc. and 3 cc.); the two controls were similarly injected. Two of the collargol rabbits were killed three and one-half hours after the last injection of hen blood for histologic study. The injections of collargol were continued, 12 cc. of 1 per cent suspension being given during the period of immunization, and all animals were bled on the seventh day following the injection with hen blood and their serums were titrated.

The results of the agglutinin titrations are given in Table 10 and of the precipitin in Table 11.

Table 10

Antigen: 1 per cent packed hen cells.
Incubated: 2 hours at 37.5 C, overnight in icebox.

Serum Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:10	++++	++++	++++	++++	++++
1:100 ...	++++	++++	++++	++++	++++
1:200 ...	++++	+++	++++	+++	+++
1:400 ...	+++	++	+++	++	+
1:800 ...	++	−	++	−	−
1:1600 ..	−	−	−	−	−

Table 11

Antigen: varying dilutions hen serum.
Incubated: 1 hour at 37.5 C.

Antigen Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:10.....	*not done*	*not done*	*not done*	*not done*	++++
1:100.....	++++	+++	++	++++	++++
1:1000.....	++++	++	−	+++	++++
1:5000	++	+	−	++	++
1:10,000.....	+	−	−	+	+

The above tables show that one of the collargol rabbits, R 3, had a precipitin titer one-hundredth as great as the other collargol rabbits

and the controls, whereas in its agglutinin titer it was one of the two highest. In the agglutinin titration, two of the collargol rabbits are about double the other rabbits.

One more collargol injection of 4 cc. of a 1 per cent suspension was given on the day following the bleeding, bringing the total amount given to 38 cc. The animals were then allowed to rest until the fifteenth day following the injection with hen blood; at this time, they were again bled and their serums titrated. Table 12 gives the agglutinin titers at this time, Table 13 the hemolysin titers and Table 14 the precipitin titers.

TABLE 12

Antigen: 1 per cent washed and packed hen cells.
Incubated: 2 hours at 37.5 C, then overnight in icebox.

Serum Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:200	++++	–	+++	–	–
1:400	+++	–	–	–	–
1:800	+	–	–	–	–
1:1600	–	–	–	–	–

TABLE 13

Antigen: 0.25 cc. 1 per cent washed and packed hen cells.
Complement: 0.5 cc. 1: 10 guinea-pig serum.
Serum: 0.5 cc. varying dilutions.
Total volume: 1.25 cc.
Incubated: 1 hour at 37.5 C.

Serum Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:500	C	C	C	C	C
1:1000	C	C	C	C	C
1:2000	C	P	C	P	P
1:4000	P	o	P	o	o

(C = complete hemolysis; P = partial hemolysis;
o = no hemolysis).

TABLE 14

Antigen: varying dilutions of hen serum.
Incubated: 1 hour at 37.5 C.

Antigen Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:500	+++	+++	+++	+++	+++
1:1000	++	++	++	++	++
1:5000	+	+	+	+	+
1:10,000	–	–	–	–	–

As at the seventh day, R 1 and R 3 were the highest in agglutinin and hemolysin titers. The precipitin titers of all were identical.

Eight days following the fifteenth day bleeding, two of the rabbits were again started on daily injections of collargol. The third rabbit, R 3, was injected intravenously with a heavy suspension of human tubercle bacilli. It is interesting to note that the first injection of collargol in R 1 and R 2 at this time produced symptoms of rather severe shock, probably to be accounted for by the fact that they had become sensitized to the egg albumen in the collargol. When R 1 and R 2 had received an additional amount of collargol equivalent to 32 cc. of 1 per cent collargol, they received on alternate days three intravenous injections (2 cc., 3 cc. and 3 cc.) of sheep blood, diluted 1:3. At this time, two rabbits that were being injected with trypan blue were added to the experiment. They had been given daily injections of 1 per cent trypan blue and at the time of being injected with the sheep blood along with the other rabbits had received 38 cc. of a 1 per cent suspension of trypan blue in the course of ten days. The collargol injections were continued until they had received since the beginning of the experiment a total amount equivalent to 78 cc. of 1 per cent collargol. One trypan blue rabbit had died and the other had received a total of 46 cc. of 1 per cent trypan blue suspension. They and the controls and the trypan blue rabbit were bled on the tenth day and the twenty-first day following the injection of sheep blood. Table 15 gives the agglutinin titers and Table 16 the precipitin titers, all at the tenth day. Table 17 gives the agglutinin titers and Table 18 the precipitin titers on the twenty-first day.

TABLE 15

Antigen: 1 per cent washed and packed hen cells.
Incubated: 2 hours at 37.5 C, then overnight in icebox.

Serum Dilutions	R 1	R 2	T. Blue	Control 1	Control 2
1:100	++	++	++++	+++	+++
1:200	++	++	++++	++	++
1:400	++	++	+++	++	++
1:800	±	+	+++	±	++
1:1600	−	−	++	−	±
1:3200	−	−	+	−	−

TABLE 16

Antigen: varying dilutions of sheep serum.
Incubated: 1 hour at 37.5 C.

Antigen Dilutions	R 1	R 2	T. Blue	Control 1	Control 2
1: 10	+++	*not done*	*not done*	*not done*	*not done*
1: 100	±	+++	++++	++++	++++
1: 500	–	++	+++	+++	++++
1: 1000	–	++	++	++	++
1: 5000	–	–	+	±	+
1: 10,000	–	–	–	–	–

TABLE 17

Antigen: 1 per cent washed and packed sheep cells.
Incubated: 2 hours at 37.5 C and then overnight in icebox.

Serum Dilutions	R 1	R 2	T. Blue	Control 1	Control 2
1: 100	+++	+++	++++	++++	++++
1: 200	+++	++	++++	+++	+++
1: 400	++	+	++++	+++	++
1: 800	++	–	+++	++	–
1: 1600	+	–	++	–	–
1: 3200	–	–	++	–	–

TABLE 18

Antigen: varying dilutions of sheep serum.
Incubated: 1 hour at 37.5 C.

Antigen Dilutions	R 1	R 2	T. Blue	Control 1	Control 2
1: 10	++	++	*not done*	*not done*	*not done*
1: 100	±	±	++++	+++	+++
1: 1000	–	–	++	++	++
1: 5000	–	–	+	+	±
1: 10,000	–	–	±	±	–

At ten days, the agglutinin titers of the collargol rabbits were practically the same as of the controls whereas that of the trypan blue rabbit was somewhat higher. As regards precipitins, both collargol rabbits were lower than the controls and the trypan blue rabbit; this was especially true of R 1 which showed one-fiftieth the titer.

On the twenty-first day, the trypan blue rabbit was again the highest with no marked difference between the collargol and control rabbits. The two collargol rabbits at this time were again by far the lowest in precipitin titer.

On the twenty-sixth day, Control 1 and R 1 were injected intravenously with 4 cc. of sheep blood, diluted 1: 2.5. They both showed

slight symptoms of shock but recovered. R 2, Trypan blue and Control 2 were then injected intravenously with 6 cc. of sheep blood, diluted 1: 2.5. All three showed severe symptoms of shock and died in three, eight and eight minutes respectively.

Discussion

The only striking difference between our collargol rabbits and the controls was the difference in precipitin titer. In the former this was consistently and markedly lower than in the latter, whereas the agglutinin and hemolysin titers were slightly higher. It will be recalled that this same difference in precipitin titer was found in the preceding experiments on guinea-pigs and this finding in two different species of animals would appear to confirm its importance. The explanation of this fact is difficult unless one can conceive, as suggested above, that soluble and particulate antigens are handled in different ways in the body. In regard to the rabbit treated with trypan blue, it showed consistently somewhat higher agglutinins than the collargol and control rabbits, but no difference in precipitin titer. This is somewhat surprising in view of the amount of trypan blue it received, which was comparable to that used by Gay and Clark.[3]

Histologic Examination of Guinea-pig Tissues

(Throughout this description of the histologic pictures in the guinea-pigs and rabbits, the word "macrophages" is used to denote the large, mononuclear phagocytes that are not fixed tissue cells; this term is used instead of the more definite one of "monocyte," "clasmatocyte" or "endothelial leucocyte" as we have no proof of the exact type of cell we are describing.)

In describing the tissues, in each instance the picture during or immediately after the collargol injection is given, then the findings several days or weeks after the cessation of the collargol injections. Each organ examined will be taken up in turn. The results represent the study of several animals in each group.

Zenker's fluid, of course, constitutes our best routine fixative for histologic work. It was used throughout this study. But Zenker's fluid so alters the collargol that the latter is no longer black or dark brown but becomes light yellow; after routine phosphotungstic acid hematoxylin it stains yellow; with eosin-methylene blue it is

green. Tissues are easily studied even with the collargol so altered but for photographic purposes the silver must be suitably blackened, One of us has devised a method for so blackening the phagocytized collargol. Sections are brought as usual through the iodine and to water; they are then treated for five minutes in a 1 per cent gold chloride solution; afterwards they are washed three to five minutes in tap water, rinsed in distilled water and treated with equal parts distilled water and stock mixture consisting of solution ammonium sulphydrate (Merck) 1 part, alcohol 3 parts. They should remain three to five minutes, following which they are washed fifteen minutes and counterstained with basic fuchsin (0.2 per cent in 50 per cent alcohol). Dehydrate, clear and mount in balsam. The method is empirical, the exact chemical changes being obscure.

Lung. In the early stages, there is a small amount of collargol present and this is contained in macrophages in the capillaries scattered throughout the lung. In the late stage, the collargol is apparently diminished in amount and what little is present is in macrophages which are in lymphatics or in the lymphoid tissue. At no time is any collargol seen in the fixed endothelium.

Spleen. In the animals killed early, the collargol is in macrophages scattered throughout the pulp and to a slight extent in the germinal centers. There is a considerable amount of collargol. In the later stages, the organ exhibited varying degrees of sclerosis (Fig. 1). The collargol is distributed in the same manner as in the early animals but apparently is diminished. In some guinea-pigs, there are varying degrees of toxic reaction and lymphoid hyperplasia but as these changes are likewise seen in the controls they cannot be considered due to the collargol.

Liver. In the early stages, the collargol occurs in the fixed endothelium lining the sinusoids throughout the organ. In the later stages, the picture is quite different. Here, the collargol is found in focal collections of macrophages, some of which are multinucleated; several of these collargol-containing macrophages are seen in mitosis; in these foci of macrophages there often are necrotic cells, and collected about these foci are collargol-free macrophages and polymorphonuclear leucocytes; some of these latter contain collargol. The collargol is likewise in macrophages in the portal connective tissue.

Kidney. No collargol is seen in either the early or late stage.

Bone Marrow. The findings in the early and late stages are essentially the same. The collargol occurs in macrophages scattered throughout the tissue. None is seen in fixed endothelium. The marrow cells in the majority of animals show myeloblastic rather than erythroblastic activity.

Lymph Node. Only a few of these are examined and in them there is a small amount of collargol in macrophages in the sinuses and in the germinal centers.

SUMMARY. In the guinea-pig, collargol is taken up in the largest amounts in the liver, spleen and bone marrow; very little occurs in the lung and none in the kidney. The only organ in which collargol can be found in fixed endothelium is the liver and even in this organ this is more true of the early than the late stages for in the late stages much of the collargol is in macrophages; these macrophages of course may be the same cells as the fixed endothelial cells which, after being filled with collargol, have become mobile instead of remaining sessile. That collargol is to a certain extent toxic is evidenced by the fact that in the late stage necrotic macrophages with a reaction about them are found in the liver. The apparent diminution in amount of collargol in the lung and spleen in the late stages as compared to the early stages may be accounted for in four ways: (1) the collargol has been excreted; (2) while the number of collargol-containing cells is decreased, the amount in each cell is increased; (3) the cells containing collargol have migrated to some other region; and (4) the collargol has been dissolved and taken up by some other organ.

HISTOLOGIC EXAMINATION OF RABBIT TISSUES

Lung. In the early stages, the collargol occurs in the fixed endothelium of the capillaries and in macrophages in the capillaries and lymphatics. In the later stages, it is found in macrophages which are collected in foci in the capillaries, in the lymph nodules and in chronic inflammatory foci in the alveolar walls and the alveoli. Also some is found in macrophages in the walls of the larger blood vessels beneath the lining endothelium. None is seen in fixed endothelium.

Spleen. Early, the collargol is in macrophages scattered throughout the pulp and to some extent at the periphery of the germinal centers (Fig. 2). Many of these collargol-containing macrophages also contain nuclear débris. (The spleens of two animals killed six

and one-half hours after their final injections of collargol show collargol in the form of a granular, metallic-appearing material free in the sinuses and in the fixed endothelial cells but its distribution in these cells suggest that it is merely passing through rather than that it has been actively phagocytized as seen in the liver). In the late stage, the collargol is found in macrophages and free giant cells (Fig. 3), often collected in foci, in the pulp and in the germinal centers. The picture suggests that the collargol in single cells is increased in amount, while the number of such cells is decreased. Many of the collargol-containing cells have phagocytized nuclear débris. No collargol is seen in fixed endothelium.

Liver. In the animals killed early, the collargol is found to some extent in the fixed endothelium lining the sinusoids throughout the liver but especially either in giant cells or in macrophages collected in foci in the sinusoids (Fig. 3). About these foci, there are collargol-free macrophages and polymorphonuclear leucocytes. A rare mitotic figure is seen in collargol-filled macrophages. In the late animals, the collargol is in macrophages, usually collected in foci, and in giant cells in the sinusoids (Fig. 7). It is found also in macrophages in the lymphatics in the portal areas. Some of the collargol-containing macrophages have also phagocytized polymorphonuclear leucocytes and nuclear débris.

Kidney. In the early stages, there is some collargol both in free macrophages and in fixed endothelium in the glomeruli. It also occurs to a slight extent in the fixed endothelium of the small capillaries between the tubules and in macrophages in lymphatics. Late, there is only a small amount of collargol in this organ and that is in macrophages in the glomerular capillaries.

Adrenal. A small amount of collargol is found in macrophages in the capillaries.

Bone Marrow. In the early stages, the collargol is found in macrophages and in the fixed endothelium lining the blood spaces (Fig. 5). Late, it occurs exclusively in macrophages and giant cells, none being found in fixed endothelium (Fig. 8). These macrophages and giant cells are arranged in foci. One such giant cell contains besides the collargol five polymorphonuclear leucocytes and one lymphocyte.

Lymph Nodes. Considerable collargol is found in fixed endothelium and circulating macrophages in early stages (Fig. 4). In late stages no definite collargol is found.

SUMMARY. In rabbits, collargol is taken up mainly in the liver, spleen, and bone marrow and to a slight extent in the lung, kidney and lymph nodes. In all organs, there seems to be a tendency for the collargol-containing macrophages to collect in foci as time goes on. The collargol gives rise to giant cell formation to a marked degree in the liver, spleen and bone marrow. In the rabbit as compared to the guinea-pig, the fixed endothelium, at least in the early stages, plays a greater part in taking up the collargol. That collargol does not interfere with phagocytosis of other material cannot be stated from our results, in spite of the fact that we often found cells containing both collargol and phagocytized cells and nuclear débris, since it is possible that such cells had phagocytized the other material before they took up the collargol. Collargol had no influence on the general condition of our rabbits as they remained perfectly well throughout and examination of their blood revealed nothing abnormal.

COLLARGOL RABBIT INFECTED WITH TUBERCULOSIS

As will be recalled, one of our collargol rabbits, R 3, after having received 37 cc. of a 1 per cent suspension of collargol, was given a heavy suspension of living human tubercle bacilli in its ear vein. This animal died thirty-nine days later. The histologic examination of its tissues are given below.

All the organs examined showed typical tuberculosis and in every organ tubercle bacilli were demonstrated by suitable staining methods.

Lung. Tuberculosis. The cells forming the tubercles are essentially free from collargol (Fig. 12). What little collargol is found occurs in macrophages collected in foci. The distribution of the collargol in these macrophages is striking and the description of it in this organ holds true of it in the other organs. The collargol in the macrophages is arranged in a spherical mass closely adjacent to the nucleus, often with a clear space in its center, in which can be distinguished the centrosomes. This arrangement corresponds closely to the "rosette" of the so-called monocyte as described by Sabin and her co-workers.

Spleen. Tuberculosis. Collargol is found in macrophages and in giant cells (Fig. 9). The cells forming the tubercles contain collargol.

Liver. Tuberculosis. The collargol occurs in macrophages in foci

(Fig. 10) and in giant cells, in the sinusoids and in the portal connective tissue. In the tubercles, a varying proportion of the cells contain collargol.

Kidney. Tuberculosis. No collargol is found in the tubercles or elsewhere.

Lymph Node. Tuberculosis. No collargol is found.

Bone Marrow. Tuberculosis. Collargol occurs in macrophages and in giant cells (Fig. 11). Two caseous tubercles are seen with free collargol in the necrotic tissue. There is little marrow activity and the majority of the cells seem to be lymphocytes and macrophages.

The interesting findings in this rabbit are two in number:

(1) The arrangement of the collargol in the macrophages. According to Cunningham, Sabin, *et al.*,[7] the monocyte phagocytizes material at the periphery of its cytoplasm, leaving its "rosette" free, whereas the clasmatocyte may arrange its phagocytized material in the "hof" next to the nucleus; following this criterion, these cells should be classified as clasmatocytes, since the collargol appears to be deposited in the very region in which Sabin located the hypertrophied rosette of the tuberculous monocyte. However, these same workers claim that the predominant cell in the formation of the tubercle is the monocyte. Now we found that many of the tubercles were formed in varying proportions by these collargol-containing macrophages which, as stated above, should be classified as "clasmatocytes." There are two possible explanations of these facts: either Sabin and her co-workers are mistaken in assigning the predominant rôle in tuberculosis to the monocyte, or collargol is arranged in monocytes in a manner different from any other material. It is interesting to note that Muller [8] had no hesitancy in classifying the cells containing collargol described in her experiments as clasmatocytes.

2. The fact that the proportion of cells forming the tubercles varied in their collargol content more or less directly with the amount of collargol in the organ. This would suggest that the cells forming the tubercle are derived purely locally.

Trypan Blue Rabbits

In order to study the distribution of trypan blue in rabbits, two additional animals were given daily injections of a filtered 1 per cent saline suspension of the dye over a period of seventeen days. The dye injected totaled 69 cc. Both animals were bled and then killed on the day following the last injection. The serums were deeply stained and the dye content was roughly estimated colorimetrically at 0.025 gm. per 100 cc. of blood. A similar titration of the trypan blue rabbit used in the above experiments showed 0.004 gm. per 100 cc. of blood. Complement titrations in these animals showed no variation from controls. Tissues examined histologically (frozen sections and rapidly prepared, alcohol-formalin fixed celloidin sections) showed trypan blue distributed as follows:

Lung. In one rabbit, the dye is in cells in the capillaries scattered throughout the lung. In the other rabbit that has an incidental bronchopneumonia, the trypan blue occurs in cells in foci about the larger blood vessels and bronchi; it also is found in cells in the exudate in the alveoli and bronchi.

Spleen. By far the major portion of the trypan blue is in macrophages in the pulp; a small amount is found in the fixed endothelium.

Liver. The dye occurs both in the fixed endothelial cells lining the sinusoids and to some extent in the parenchymatous cells.

Kidney. The trypan blue occurs exclusively in the convoluted tubules. None is found in the glomeruli or endothelium elsewhere.

Lymph Node. Both the macrophages in the sinuses and the fixed endothelial cells lining the sinuses contain trypan blue.

Bone Marrow. The trypan blue is found in both the fixed endothelial cells lining the blood spaces and in macrophages scattered through the hematopoietic tissue.

Summary and Conclusions

1. Collargol injected repeatedly intravenously in guinea-pigs produced the following effects on immunologic responses:
   (1) depression of precipitin formation;
   (2) depression or stimulation of agglutinin production; and
   (3) protection against anaphylactic shock.
2. Rabbits treated in a similar manner showed the following:
   (1) depression of precipitin production;

(2) slight or no stimulation of agglutinin or hemolysin production; and

(3) no protection against anaphylactic shock.

3. Histologic studies indicate that blockade of fixed endothelium is not obtained with either collargol or trypan blue except in the liver and to slight extent in lymph nodes and bone marrow. Once attained, continued injection is necessary to keep the blockade advanced over the segregation of blocked cells, and to maintain block a considerable amount of circulating colloidal material is needed. In view of this fact it is difficult to assert categorically that resultant alterations in antibody production are due to endothelial blockade.

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DESCRIPTION OF PLATES

PLATE 75

FIG. 1. Spleen, late guinea-pig; sclerosis. × 250.
FIG. 2. Spleen, early rabbit. × 250.
FIG. 3. Liver, early rabbit; granules in liver cells not collargol. × 250.
FIG. 4. Lymph node, early rabbit. × 1000.

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FIG. 5. Bone marrow, early rabbit. × 500.
FIG. 6. Collargol-filled giant cell, spleen, late rabbit. × 750.
FIG. 7. Liver, late rabbit; granules in liver cells not collargol. × 250.
FIG. 8. Bone marrow, late rabbit; these marrows were not uniformly aplastic. × 500.

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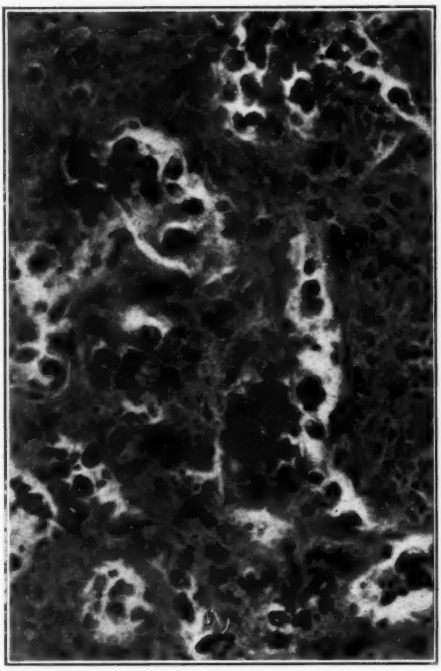
FIG. 9. Spleen, tuberculous rabbit. × 750.
FIG. 10. Liver, tuberculous rabbit. × 750.
FIG. 11. Bone marrow, tuberculous rabbit. ×750.
FIG. 12. Lung, tuberculous rabbit. × 750.



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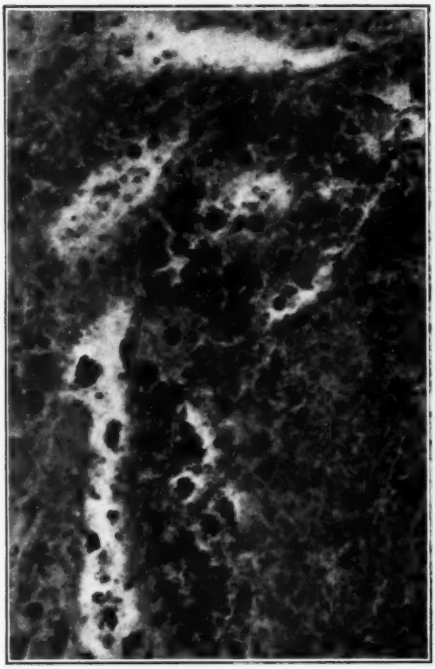

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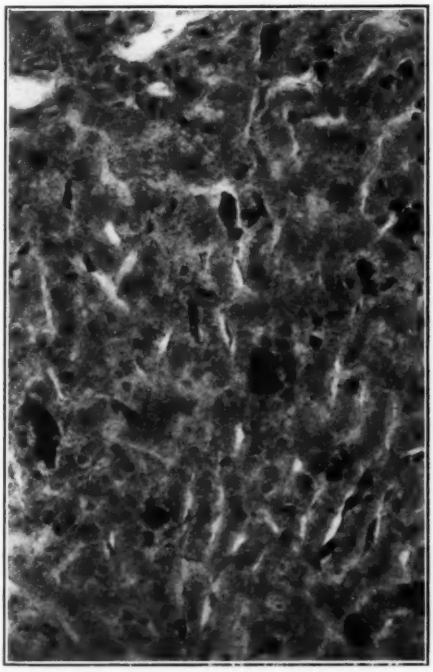

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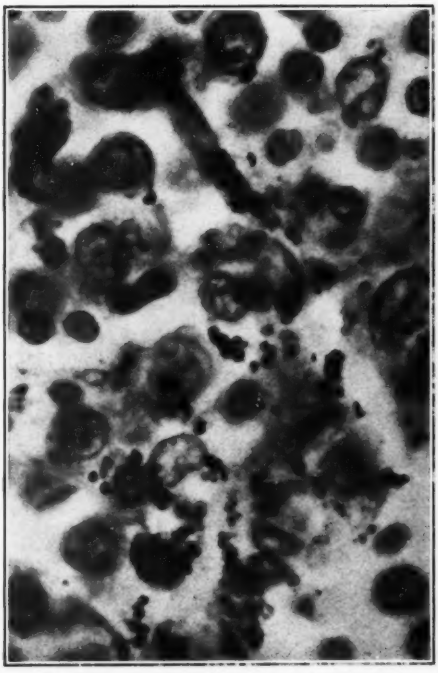

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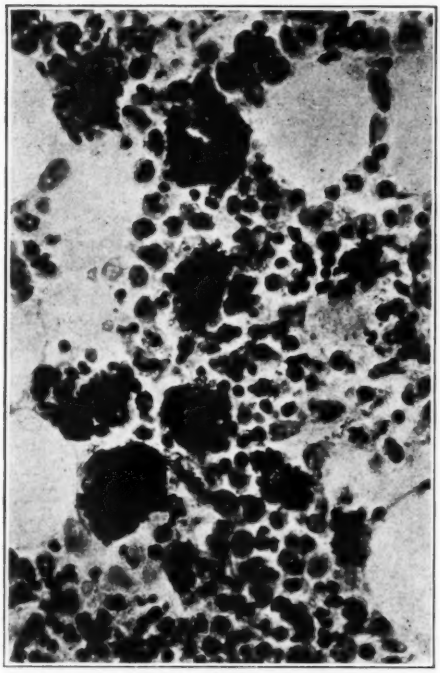
Stewart and Parker

So-called "Endothelial Blockade"



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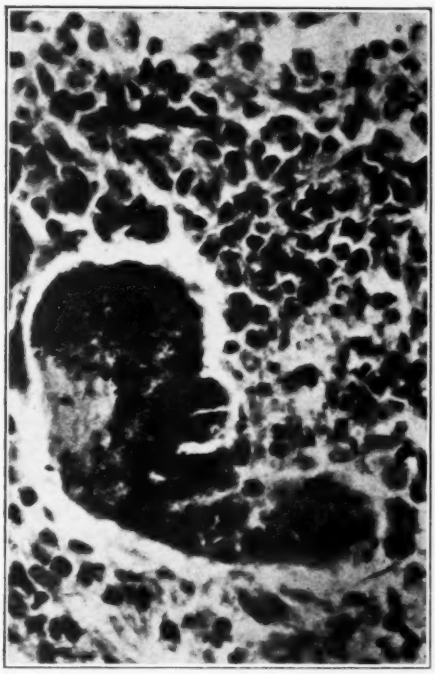

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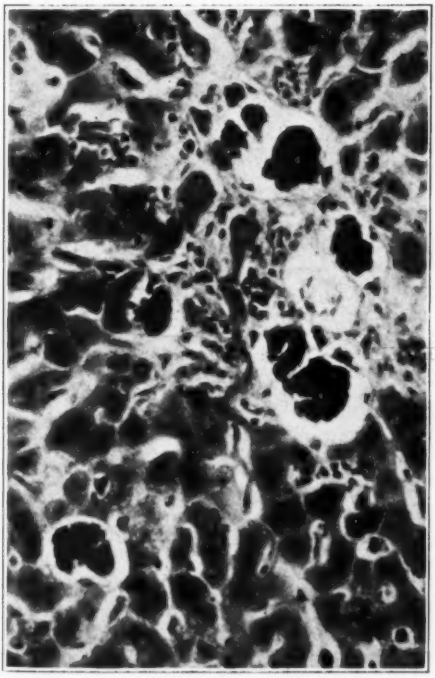

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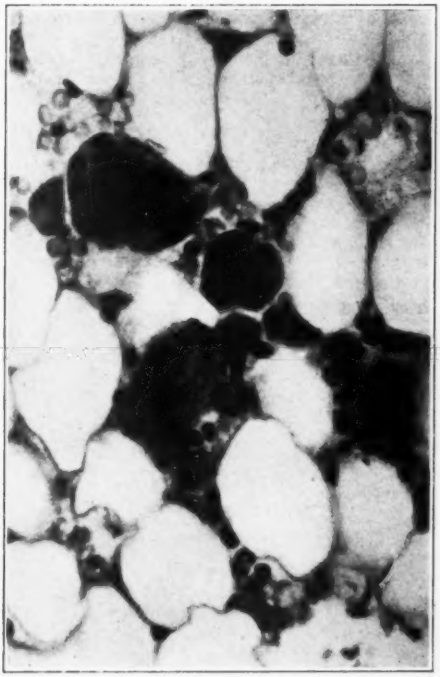

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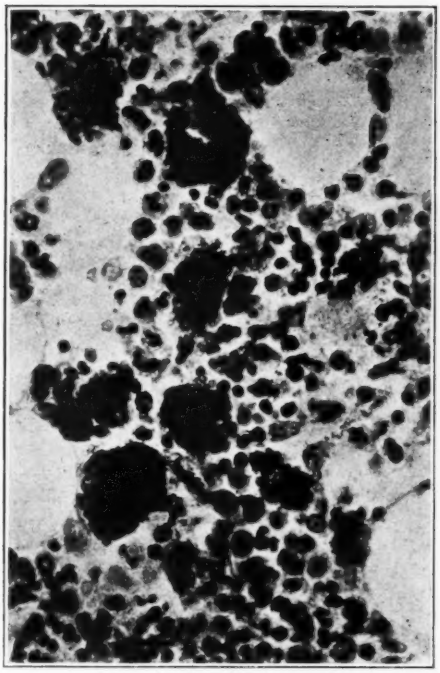
Stewart and Parker

So-called "Endothelial Blockade"



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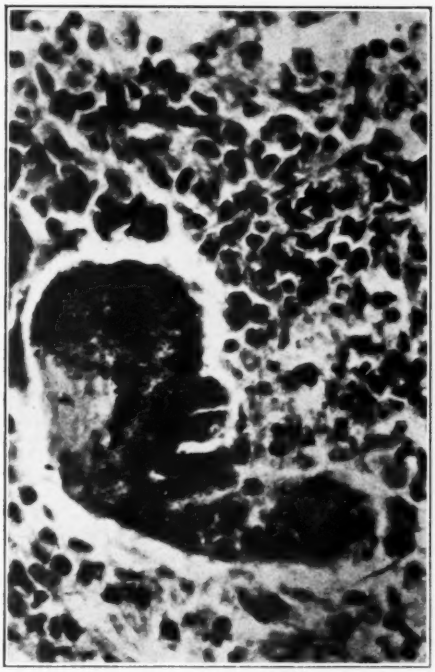

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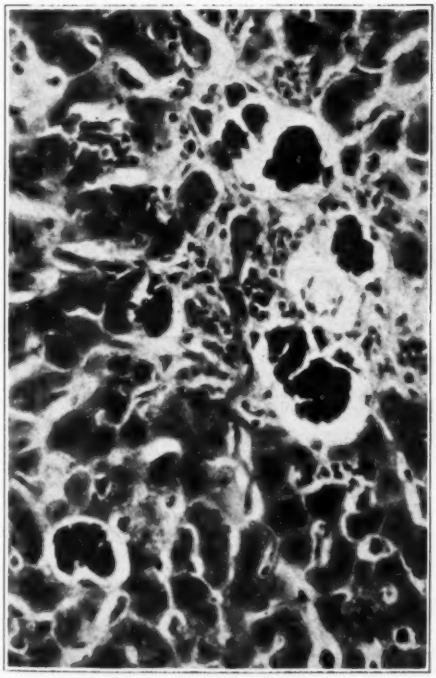

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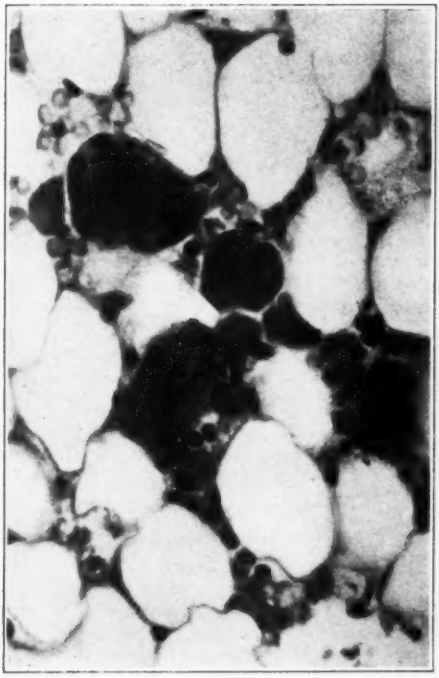

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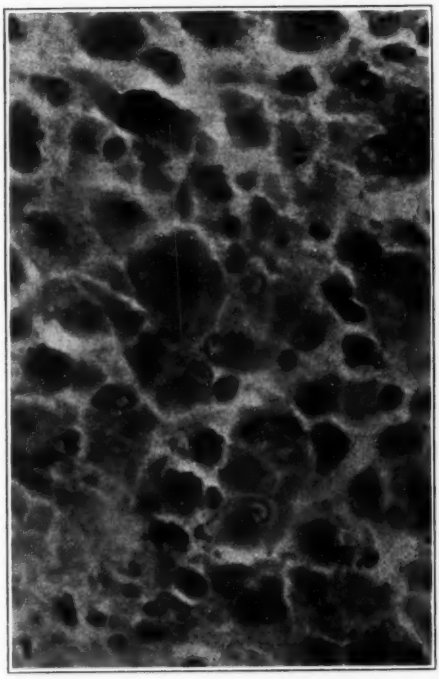

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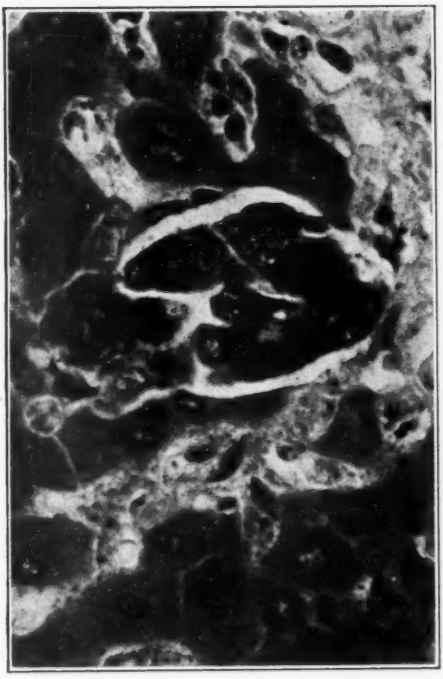

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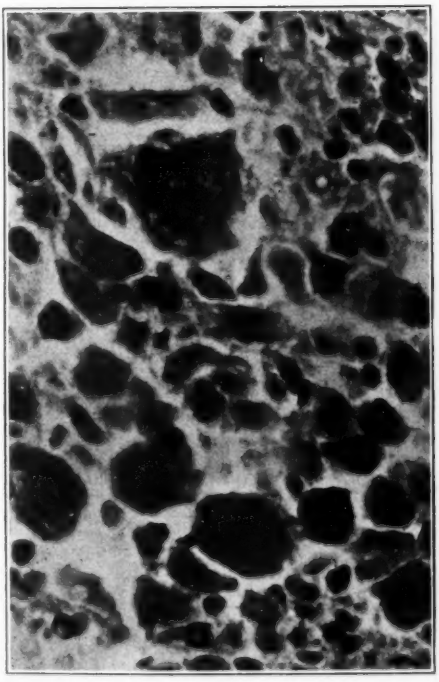

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So-called "Endothelial Blockade"

CASES OF RENAL INFECTION IN PULMONARY TUBERCULOSIS

EVIDENCE OF HEALED TUBERCULOUS LESIONS *

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Renal tuberculosis as seen in clinical practice comprises, as stated by Caulk,[1] 30 per cent of all surgical lesions of the kidney. These cases are usually diagnosed at a date when there is no apparent clinical manifestation of tuberculous lesions in any other part of the body and hence are looked upon as primary lesions. The extensive destruction of kidney tissue as found in such cases has led to the belief that tuberculosis of the kidney is a progressive destructive lesion which does not heal. Because of this interpretation it is now a common belief that whenever tubercle bacilli are found in one ureteral urine and not in the other, the kidney from which the bacilli are excreted should be removed regardless of the size of the lesion. The truth or falsity of this belief, that renal tuberculosis does not heal, is the main consideration of this paper.

Renal tuberculosis with cavitation, that is renal phthisis, would appear to correspond to the stage in pulmonary tuberculosis where cavitation is present. They are both advanced stages of the disease. Since it is a well established fact that all cases of tuberculous infection of the lung do not go on to cavitation, it would seem quite probable that the same fact would apply to tuberculous infection of the kidney. If the pathologic study of pulmonary tuberculosis were limited to cases with cavitation it would not give a true conception of the disease in its entirety as it occurs in the lung. It would seem plausible that this might also be the case in tuberculous infection of the kidney.

With the above consideration in mind, it was decided to study the kidneys from patients dying of pulmonary tuberculosis. The cases chosen gave no clinical symptoms of renal involvement. No case of renal phthisis was included. Thirty cases were selected all of which showed active tuberculous lesions of the lung with caseation. The

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immediate cause of death was advanced pulmonary tuberculosis in twenty-seven cases, tuberculous meningitis secondary to pulmonary tuberculosis in two cases and streptococcal abscesses of the brain with advanced pulmonary tuberculosis in one case. The ages range from 18 months to 70 years.

Both kidneys were examined in fourteen cases. In sixteen cases only one kidney was available. In all, forty-four kidneys have been examined.

The technic of gross examination used was as follows. The kidneys were split open lengthwise and fixed *in toto* for forty-eight to ninety-six hours in 10 per cent formalin. They were then separated into two halves and each half was cut into strips from two to three millimeters in thickness. Portions of the kidney tissue which appeared abnormal were cut out for microscopic study. In those cases where definite tuberculous lesions were numerous, the smaller lesions only were saved. All areas where there was a depression in the kidney surface were also removed for histologic examination. With this technic it was much easier to be certain of small lesions in the kidney tissue than in fresh unfixed organs. The technic is admittedly faulty in that without doubt many small lesions were overlooked. But the labor involved in proper histologic preparations of entire kidneys necessitated the removal of small blocks of tissue for microscopic study.

The blocks of tissue were embedded in paraffin in the usual manner. The tissue was then serially sectioned and mounted on glass slides. Every third slide was stained for tubercle bacilli by the use of Verhoeff's carbol-fuchsin. The sections were first overstained with hematoxylin and then placed in cold carbol-fuchsin over night. One per cent hydrochloric acid in 80 per cent alcohol was used as the discharging fluid, the sections being treated for five to ten minutes. The remainder of the sections were stained with hematoxylin and eosin.

In this study approximately 100,000 sections have been carefully examined. Serial sections were found invaluable as entire lesions could be followed through. In the case of scars it was thus possible to determine whether the reparative process simply surrounded a lesion incompletely healed or whether the lesion was completely healed. As high as fifty sections in some of the scars were carefully examined for the presence of tubercle bacilli. By this method it was

also possible to demonstrate tubercle bacilli in lesions where single sections would have yielded negative results. In some lesions the bacilli were so scarce that but one bacillus could be found in ten sections. In other lesions a single section would show hundreds of the organisms. With this technic one was able to determine whether the lesion was entirely cortical, entirely medullary or cortico-medullary. It was possible also to find many microscopic lesions that would have escaped detection by any other technic.

In all, 367 definite tuberculous lesions were studied. These lesions varied from small tuberculous abscesses to lesions in which the only evidence of their tuberculous nature was revealed by the presence of an occasional giant cell or of a microscopic mononuclear tubercle. Tubercle bacilli were found in 265 of these lesions. Many of the small lesions were not stained for tubercle bacilli. Others were stained but no tubercle bacilli were found and in these cases the only evidence that the process was tuberculous in nature was the presence of one or more giant cells. There was great variation in the number of tubercle bacilli in the different cases. In one case fifty-four separate lesions were studied and tubercle bacilli were numerous in every lesion. In another case twenty-nine separate lesions were studied and tubercle bacilli were found, after prolonged search, in but one lesion.

The distribution of the lesions within the kidney tissue was as follows. Cortical lesions numbered 277 or a fraction over 75 per cent; medullary lesions were present forty times or about 11 per cent; and cortico-medullary lesions numbered 50 or about 13 per cent.

A study of the point of origin of the lesions proved of interest. For this study the smaller or microscopic lesions were relied upon, as the larger lesions involved too much tissue to make it possible to determine with assurance the exact point of origin. The smaller lesions were of two main types. The most common of these types was of vascular origin within the capillary tuft of a glomerulus, within a capillary between the convoluted tubules or within a capillary between the collecting tubules in the pyramids. Of these points of origin the most common was within a glomerulus and the least common in the pyramid. In the early glomerular lesion several instances were found in which one half of the glomerulus was involved while the remainder was normal.

The second type of small lesion had its origin within the lumen of

tubules. These lesions were found with about equal frequency in the lowest point of the loop of Henle and in the collecting tubules in the pyramid. They always subtended an ulcerating tuberculous lesion of some portion of the kidney substance. It appears that this type of lesion is always secondary to a vascular lesion which has developed to a point where destruction of tissue has supervened and tubercle bacilli have been discharged into the lumen of a tubule. In some instances the primary vascular lesion was a small tuberculous abscess involving but one glomerulus.

The points of prime importance in this study were the search for scars in the kidney tissue and the study of them. At the outset the difficulty of the interpretation of scars in the kidney was realized. Scars as the end result of infarction are so typical that their interpretation affords little difficulty. Scars occurring in kidneys where there was evidence of atherosclerosis were disregarded in the study. The healed lesion of infections other than tuberculosis would appear to be impossible to separate from healed tuberculous lesions in which caseation had been absent or at most was scanty. In other words, in a considerable portion of healed tuberculous lesions one could hardly expect to find anything which would label it as tuberculous in nature. This certainly is true in other organs of the body. Sclerosed glomeruli even in young individuals where there was no evidence of atherosclerosis were disregarded in this study, although it was felt that in all probability at least some of them represented the healed stage of a tuberculous process within the glomerulus.

Of the thirty cases studied, two gave no evidence of tuberculous lesions or scars. In twenty-two cases, definite tuberculous lesions were found and of these cases fourteen showed scars also. The remaining six cases showed scars only. Of the twenty cases which showed scars, three showed definite atherosclerotic lesions. Two of these sclerotic cases had definite tuberculous lesions and the third did not. The seventeen cases which did not have atherosclerotic lesions were also free from infarction except in connection with some of the larger tuberculous lesions. Not all of the scars were tabulated, but the following was the distribution of 100 of these lesions: cortical, 80; medullary, 14; and cortico-medullary, 6. From this it will be seen that the scars were distributed in the different renal regions in approximately the same proportion as were the active lesions.

The size and histologic picture of these scars varied considerably. Most of the scars were microscopic in size. Thirty-one scars of macroscopic size were found and the largest of these measured 3 mm. in diameter. All of the lesions showed destruction of kidney tissue, which varied from very slight to a rather large area of destruction, with a replacement of the area by connective tissue. In some of the larger lesions lymphocytic infiltration was marked but this was not constant, for many of the lesions showed no lymphocytic infiltration except perhaps an occasional lymphocyte in the tissue around the periphery. The density of the connective tissue varied greatly, being very compact in a few of the scars and of a rather loose texture in the majority of the lesions.

In all of the larger lesions some normal kidney tissue was present within the scarred area. If the lesion were cortical, normal glomeruli and convoluted tubules were present, while in the medullary region normal collecting tubules were present. These normal structures were always much fewer in number in these areas than in the surrounding normal tissue. At the periphery of the lesion it was common to find dilated tubules filled with hyaline material. This condition was interpreted as being due either to pressure upon a tubule as it passed through the scarred area or to the destruction of the portion involved in the scarred area with the peripheral portion remaining functional. In some of the larger cortical lesions a rare sclerosed glomerulus was found. Four scars contained small irregular particles which resembled bits of old caseous material.

One case in this series will serve to illustrate a condition which is probably quite common among tuberculous individuals. The patient was a man of about 35 years who entered the sanatorium at Mt. McGregor, New York, eight years before his death, as a case of far advanced pulmonary tuberculosis. For two years he underwent rigid sanatorium treatment. At the end of this time he was sufficiently improved to run the print shop at the sanatorium. This work he continued uninterruptedly until two weeks before his death. Monthly examinations were made during these six years and there was no evidence of a recrudescence of the pulmonary lesion, though his sputum remained positive. He never had any clinical symptoms pointing toward renal involvment. About two weeks before his death he developed a hemiplegia following a rather severe bronchitis.

Necropsy of the above case showed death to be due to two streptococcal brain abscesses. One lung showed an old fibroid phthisis and the other multiple old tuberculous lesions. There were many old tuberculous lesions in the spleen and liver.

Both kidneys showed tuberculous lesions. Six thousand sections from these kidneys were examined. There were 33 tuberculous lesions of which 25 were cortical, 5 were cortico-medullary and 3 were medullary. Tubercle bacilli were extremely hard to find and were demonstrated in but nine lesions. Only six lesions showed areas of caseation. In many of the lesions the only evidence suggesting tuberculosis was the presence of one or more giant cells. Besides these lesions there were numerous scars such as have been described above. These scars were present in the cortex and in the pyramids. Two lesions which in gross appeared to be caseous areas proved on section to be old caseous material, containing numerous cholesterin crystals, with a fibrous wall infiltrated with lymphocytes and an occasional mononuclear leucocyte completely surrounding it. Over 200 sections in these two lesions were carefully searched for tubercle bacilli. Two bacilli were found in one lesion and none was found in the other. These lesions at a previous date were undoubtedly active, ulcerating, caseating lesions. When examined, while not completely healed, they were so thoroughly walled off as to be innocuous to the individual. It would seem highly improbable that tuberculous kidneys such as these would excrete tubercle bacilli in the urine at this stage.

As stated above, both kidneys were examined in fourteen cases. Two of these cases were entirely negative as far as my examination went. One case showed only scars in both kidneys. The remaining eleven cases had tuberculous lesions in both kidneys. In several cases the lesions were larger in one kidney than in the other. The reason for this difference is not certain, although the chance of bacillary dosage would appear the most logical explanation.

Small benign tumors were encountered in six of the thirty cases. These tumors were multiple. In three cases papillary adenomas of the cortex were present. Two cases showed fibromas of the medullary region. One case had both types of tumor present. Microscopically, neither type of tumor could be confused with scars or active tuberculous lesions, as they were encapsulated and had definite tumor architecture.

As a control to the above series of tuberculous kidneys, the kidneys from twenty-two necropsies on non-tuberculous individuals were examined by the same technic. Only sufficient sections were examined to ascertain the nature of the pathologic lesions found. It was not deemed necessary to follow the lesion through in serial section. The age range of the cases was from 6 months to 76 years. The list of diseases is as follows: bronchopneumonia, 3; pernicious anemia, 2; ascending bilateral pyelonephritis, 3; endocarditis, 3; cardio-renal, 3; malignancy, 4; chronic glomerulonephritis, 1; gummas of liver with ruptured esophageal varix, 1; and *Staphylococcus aureus* septicemia, 2.

The lesions observed in the kidneys of these cases were as follows: atherosclerotic scars, 7; infarcts (cases of endocarditis), 2; chronic glomerulonephritis, 1; acute infectious nephritis with abscess formation, 5; small cortical scars, 2 (both were cases of endocarditis); fibroma (medullary), 1; papillary adenoma (cortical), 2; and negative, 8.

The kidneys showing infectious lesions and scars were more thoroughly studied than the remaining cases. The cases of *Staphylococcus aureus* infection were in young individuals and the distribution of the lesions in the kidney tissue was very similar to the distribution in tuberculous infection. Cortical lesions were by far the most common, but cortico-medullary and medullary lesions were also present. The lesions were bilateral in both instances.

The examples of bilateral pyelonephritis occurred in adult males and were the result of obstruction in the lower genito-urinary tract. One of these cases showed rather extensive atherosclerotic scars. A second had slight diffuse scarring but no healed lesions similar to those seen in the tuberculous cases and the third was devoid of scars.

The two kidneys which showed cortical scars were in young adults with endocarditis. There was no evidence of atherosclerosis. All of the scars observed were cortical and appeared to be healed infectious lesions. These lesions were not numerous. They were indistinguishable from many of the scars found in the tuberculous kidneys.

Discussion

In order to interpret the pathologic picture presented in any tuberculous process it is essential to have in mind the pathogenesis of the tuberculous lesion. For a full consideration of the cytologic reaction

and its meaning in tuberculosis the reader is referred to two articles now in process of publication. These articles will appear in the American Journal of Pathology and they express my interpretation of the tuberculous process. A very brief résumé of this interpretation will be given here to aid in clarifying the opinions given below.

The first and typical reaction to the tubercle bacillus is the "epithelioid" or mononuclear tubercle. If the individual has high resistance, caseation does not ensue and the hyperplastic type of tuberculosis is produced. In such cases, if the tubercle bacilli are destroyed, as undoubtedly they often are, the end result is a small scar.

If the individual is unable to cope with the infection, polymorphonuclear leucocytes are attracted to the injured tissue and an abscess is formed. In case this area is so situated that the necrosing tissue can be discharged to the outside, ulceration or cavitation occurs. It is at this stage of the process that tubercle bacilli occur in the sputum or in the urine. In case this discharge cannot occur, caseation supervenes through the death and disintegration of all cells within the abscessed area. Subsequent to caseation the polymorphonuclear leucocytes do not appear to be further attracted to the lesion.

Following caseation, that is in the reparative stage of the disease, the mononuclear leucocytes and lymphocytes are attracted in large numbers. It is in this stage of the process that giant cells appear, so that whenever giant cells occur in a tuberculous lesion it is definite evidence of a reparative reaction on the part of the host. As a reparative process goes on toward completion the need for mononuclear leucocytes and lymphocytes becomes less and less until the end product is a scar with nothing pathognomonic of the etiologic factor which was the responsible agent in the infection.

If the above interpretation of the tuberculous process from its inception to the healed stage is, in the main, correct, then the interpretation of the tuberculous lesions as found in the kidney can be set forth with a fair degree of accuracy. From this study it appears that the tuberculous lesion in the kidney does not differ from that in other organs and tissues of the body, providing the differences in histologic and anatomic structure are borne in mind. As far as can be determined, the same types of cells participate in the defensive and reparative processes of the tuberculous lesion in all tissues and organs.

It was not uncommon in this study to find a great variety of tuberculous lesions in a single kidney. Mononuclear tubercles, tuberculous abscesses, areas of caseation, scarred areas infiltrated with lymphocytes and with one to many giant cells present, and scars devoid of lymphocytic or mononuclear leucocytic infiltration have all been observed in one organ. From this is would appear that the individual had had, at intervals, showers of tubercle bacilli in the blood stream and these showers have been followed by the development of tuberculous lesions in the kidney. The pathologic processes found in such organs represent, then, lesions of different age and severity, and the scars represent the healed stage in an area where the tubercle bacilli have been successfully overcome. The fact that out of twenty-two cases with definite tuberculous lesions, twelve with no evidence of atherosclerosis showed scars, whereas out of twenty non-tuberculous cases, in only two with no evidence of atherosclerosis were scars found, leads one to the logical conclusion that at least a portion of these scars, and in all probability a goodly portion, represent healed tuberculous lesions.

The cases cited above did not show completely healed renal tuberculosis. At least three of the cases would have gone on to extensive destruction of kidney tissue, if one may forecast this condition from the numbers of tubercle bacilli present and the severity of the inflammatory process. On the other hand, five cases out of thirty studied showed scars only. These cases showed no evidence of atherosclerosis. If one grants that at least a portion of these scars represent healed tuberculous lesions, then there is definite evidence that, under favorable circumstances, renal tuberculosis can heal completely.

A fact of some surprise in this study was that none of the cases presented clinical manifestations pointing toward renal involvement. A study of the urine for tubercle bacilli was not made. Judging from the presence of inflammatory exudate and tubercle bacilli in the lumen of tubules, six of the cases or 20 per cent should have had bacilluria. Brown [2] reported 10 per cent positive urines in 104 cases and Hobbs [3] reported 6 per cent in 100 cases where there were no clinical manifestations of renal involvement. So it is apparent that renal tuberculosis with bacilluria can exist without causing clinical manifestations. The authors here quoted, and others, believe that bacilluria is at times encountered in the absence of kidney lesions.

My belief is that "excretory bacilluria" does not exist without ulcerative tuberculous lesions in the kidney. That these lesions are often microscopic and are many times overlooked is in all probability the reason for the belief in "excretory bacilluria." Such lesions may involve but a part of one glomerulus.

If one may judge by the absence of inflammatory exudate and bacilli in the lumen of tubules at least one-half of the cases in this series would not have shown tubercle bacilli in the urine. From this it would seem that renal tuberculosis can exist without bacilluria being present.

The common occurrence of tuberculous lesions in the kidney in cases of pulmonary tuberculosis was unexpected. This report shows a very high percentage. If a much larger number had been examined the percentage might have been lower. A probable explanation of the findings is that the examination of the kidneys have been much more thorough and that serial sections have revealed many lesions unsuspected on gross examination. If the findings in this study represent the true facts, it is apparent that every case of progressive pulmonary tuberculosis is a potential candidate for renal infection. It would also seem that cases of renal tuberculosis are secondary to some other tuberculous focus, usually pulmonary, in the body and that the infection is hematogenous. That the infection is hematogenous is indicated by the preponderance of cortical lesions.

Caulk [1] states that there is no authentic case on record of healed tuberculosis of the kidney. I have not been able to find such a case recorded. Hobbs [3] states in his article that he found an occasional scar and gives an illustration of the lesion. The illustration appears more like the fibromas I have encountered than like the scars I have described above. It would appear that the reason for no recorded case of healed renal tuberculosis is that the majority of cases studied have been of renal phthisis and that where tuberculosis of the kidney has been observed in the routine of a necropsy its occurrence has been automatically recorded without a thorough systematic study of the kidney with a view to determine the possibility of healed lesions in the same kidney. Renal phthisis does not afford suitable material for the study of healing of the tuberculous process, as it is an advanced progressive lesion. Healing of a cavitated kidney probably

does not occur. There is reason to believe, however, that under suitable circumstances such lesions may be clinically arrested.

It is now known that tuberculous lesions of the lung, of the intestine and of other tissues do heal. With the evidence given above regarding scars in the kidney in cases of tuberculosis, it would seem illogical to maintain the attitude that renal tuberculosis never heals.

The purpose of this paper is to present as completely and as concisely as possible the pathologic side of renal tuberculosis. The clinical side is another study but it would seem plausible that the more nearly the pathology is understood, the more sane will be the course of clinical treatment advised. The following pathologic facts are emphasized. The presence of tubercle bacilli in ureteral urine establishes the diagnosis of renal tuberculosis but not of renal phthisis. The absence of tubercle bacilli in the urine does not rule out tuberculosis of the kidney. Renal tuberculosis is of hematogenous origin and, as far as this study goes, when it occurs it is always bilateral. Tuberculous lesions of the kidney heal. Renal tuberculosis can exist without clinical manifestations.

With the above discussion in mind it would seem advisable to establish the following facts before nephrectomy for renal tuberculosis is undertaken: (1) evidence of considerable destruction and cavitation of the kidney; (2) the presence of tubercle bacilli in the urine on several examinations; (3) the absence of tubercle bacilli in the urine from the opposite kidney on several examinations; and (4), the failure of treatment on the same basis as for pulmonary tuberculosis to arrest the condition.

I have not felt it advisable to quote extensively from the literature on renal tuberculosis in this article. For the more important articles on the subject the reader is referred to the bibliography in a previous article by Dr. Sasano and myself.[4]

Conclusions

1. Renal tuberculosis is common in advanced pulmonary tuberculosis, twenty-two out of thirty cases.

2. Renal tuberculosis is hematogenous in origin, 75 per cent of the lesions being cortical.

3. Bilateral infection was the rule in every case in this series in which both kidneys were examined and tuberculous lesions were present.

4. Tuberculous lesions of the kidney heal. Scars were present in seventeen out of thirty cases. Twelve of these cases also had tuberculous lesions.

5. Serial sections are invaluable in such a study.

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2. Brown, L. *J. A. M. A.*, 1915, lxiv, 886.
3. Hobbs, F. B. *Tubercle*, 1923, v, 57 and 105.
4. Medlar, E. M., and Sasano, K. T. *Am. Rev. Tuber.*, 1924, x, 370.

DESCRIPTION OF PLATES

PLATE 78

FIG. 1. A tubercle in a glomerulus. The lower part of the glomerulus is not involved. The tubercle shows evidence of injury to the tissue but no caseation. The capsule and pericapsular tissue is involved to the left. Sclerosed glomeruli showing only partial involvement of the capillary tuft such as this have been found. × 350.

FIG. 2. A small tuberculous abscess between the tubules in the cortex. The inflammatory exudate consists largely of polymorphonuclear leucocytes. The tubules to the left contain inflammatory exudate and tubercle bacilli. There is no evidence of caseation. The lesion is microscopic in size. × 350.

PLATE 79

FIG. 3. A tubercle in a glomerulus undergoing caseation at its periphery. This is in reality a small tuberculous abscess. Note the normal portion of the glomerulus above. × 350.

FIG. 4. A caseous lesion undoubtedly arising in the glomerulus in the center of the field. The dark portion is closely packed with "nuclear dust." This is a later stage of a lesion like Fig. 3. × 350.

PLATE 80

FIG. 5. A microscopic mononuclear tubercle in the tissue to the right of the glomerulus. × 350.

FIG. 6. A scar in a position similar to the tubercle in Fig. 5. Such scars were quite frequently found and represent what I believe to be healed tubercles which have not gone on to caseation. Note the fibrous thickening of the capsule of Bowman. × 350.

FIG. 7. A giant cell tubercle. This was the only evidence of tuberculosis in a series of 300 sections from a block of kidney tissue. Other larger tuberculous lesions were found in this same kidney. This giant cell extended through 20 sections 10 microns thick. It represents a tubercle which has undergone necrosis or caseation, probably the latter, and which is now undergoing repair. × 350.

PLATE 81

FIG. 8. A large cortical scar of macroscopic size with normal glomeruli and tubules in it. There is marked fibrosis and some lymphocytic infiltration. No evidence that this lesion was tuberculous was found but there were low grade tuberculous lesions in the same kidney. Note the dilated tubules above. This is a healed infectious lesion and the probability is in favor of it being a healed tuberculous lesion. × 100.

FIG. 9. A higher power of Fig. 8, showing a normal tubule, scar tissue and some lymphocytic infiltration. × 350.

PLATE 82

FIG. 10. A medullary scar of macroscopic size. In other sections this scar contained two dilated collecting tubules. × 100.

FIG. 11. A tuberculous lesion. The only evidence is three giant cells and a small mononuclear tubercle above. After prolonged search one tubercle bacillus was found. For all practical purposes this lesion is healed though pathologically it is not healed. × 200.

FIG. 12. A tuberculous lesion. The only evidence in over 100 sections was these two giant cells. No tubercle bacilli were found. × 350.

PLATE 83

FIG. 13. A large cortical scar from a tuberculous case. No active tuberculous lesions were found in this case. × 100.

FIG. 14. An old caseous area walled off by fibrous tissue. This is one of the lesions mentioned in the text. No tubercle bacilli were found after very careful search. × 100.

PLATE 84

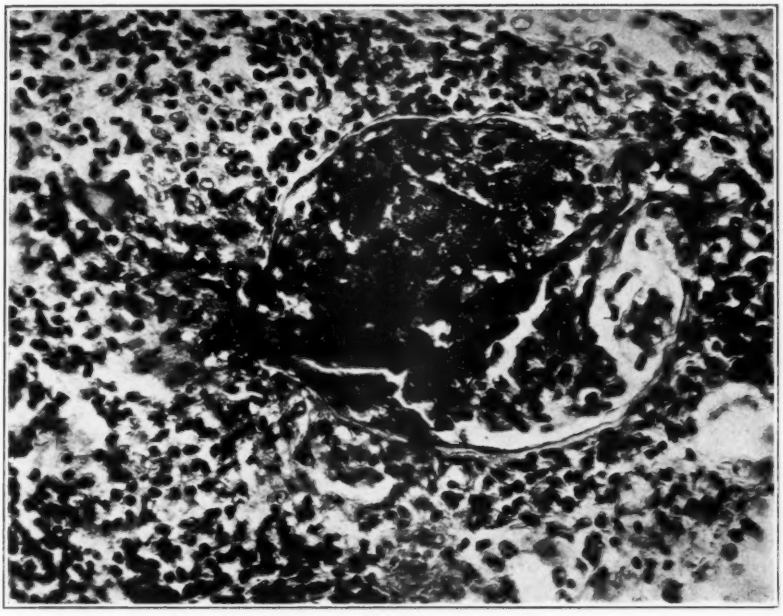
FIG. 15. A large cortical scar. The only evidence of its having been tuberculous is a giant cell at the left. × 200.

FIG. 16. A large medullary scar of macroscopic size. Note the normal tubules within the scarred area. Some of the tubules are considerably dilated. There are also small irregular hyaline masses in the tissue which I take to be bits of old caseous material. Four such scars were found in three cases of this series. × 200.

PLATE 85

FIG. 17. A medullary fibroma. Compare the architecture of the tumor with that of the scars in Fig. 9, 10, 12, 16 and 18. × 100.

FIG. 18. Dense fibrous scar. This represents a healed caseated tuberculous lesion. The sclerosed glomeruli shown above are typical of such lesions commonly seen in tuberculous kidneys which show no evidence of atherosclerosis. × 200.



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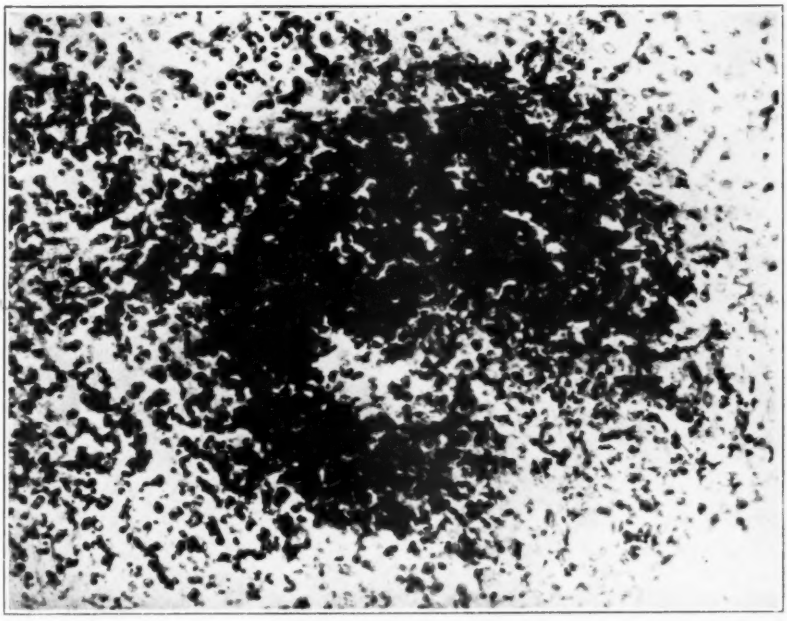

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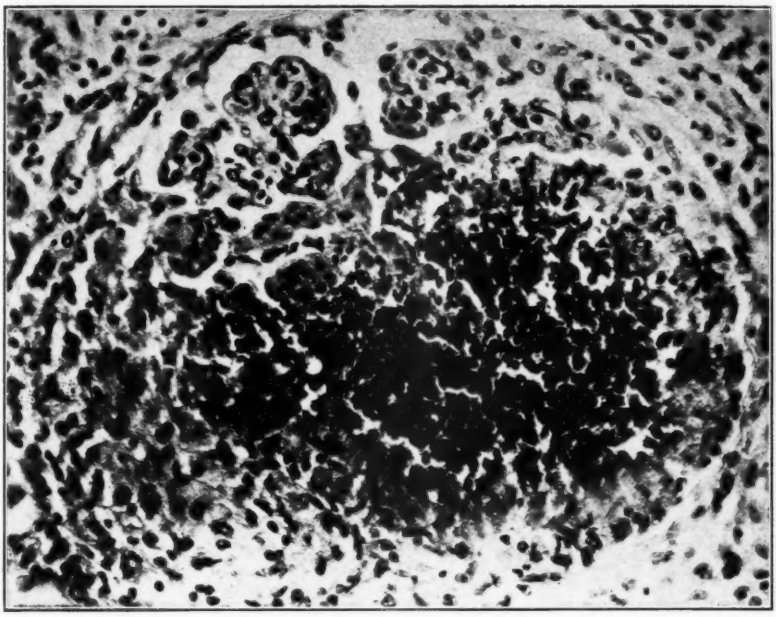
Medlar Renal Infection in Pulmonary Tuberculosis



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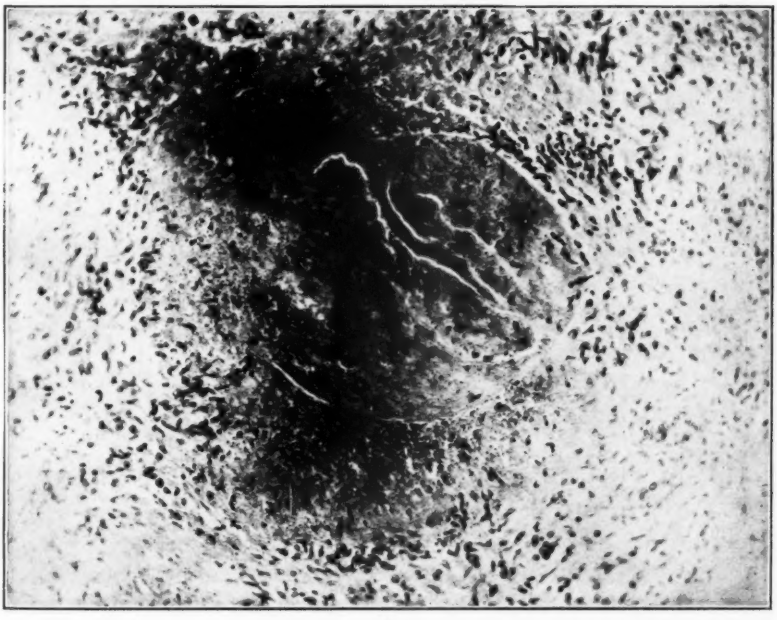

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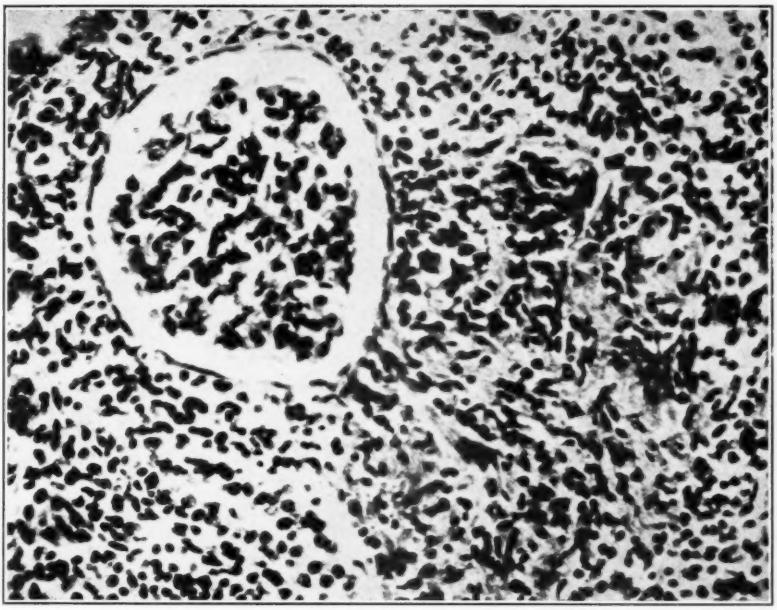
Medlar Renal Infection in Pulmonary Tuberculosis



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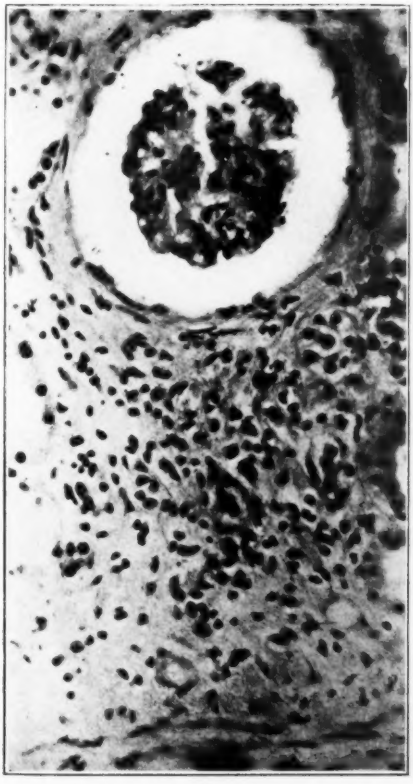

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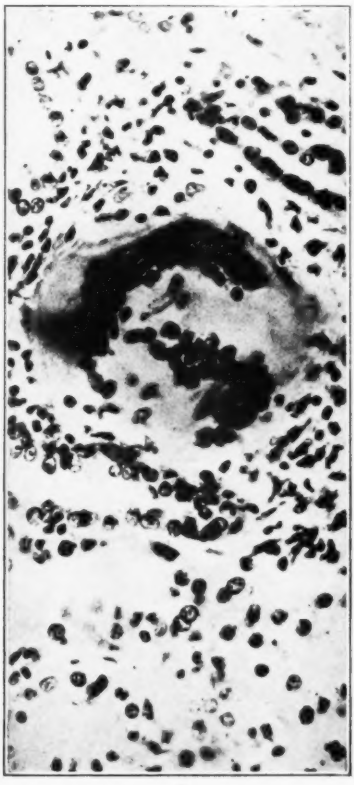

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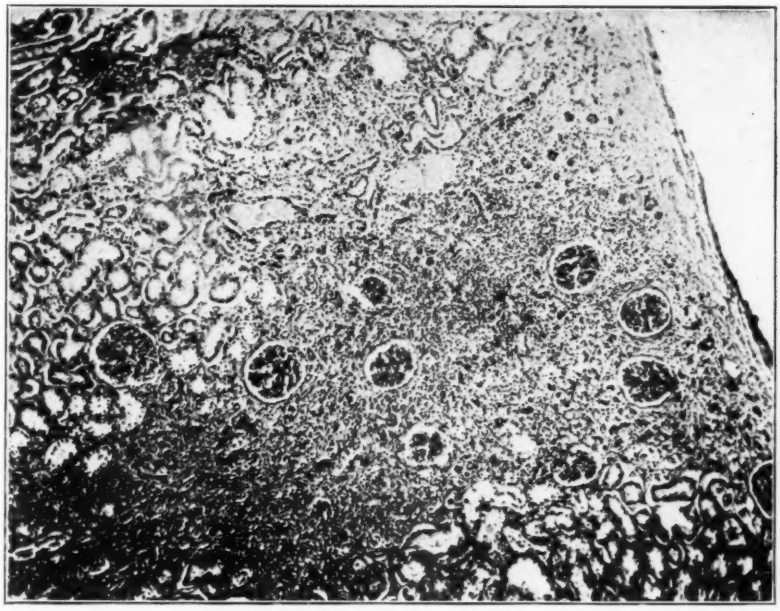
Medlar Renal Infection in Pulmonary Tuberculosis



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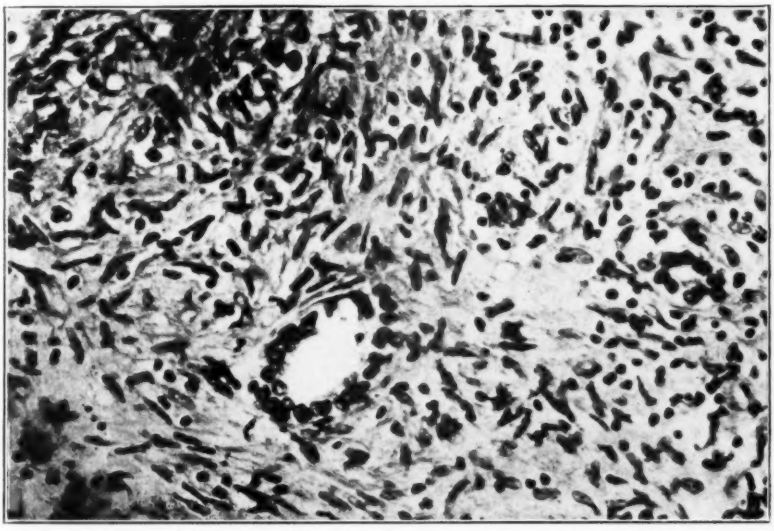

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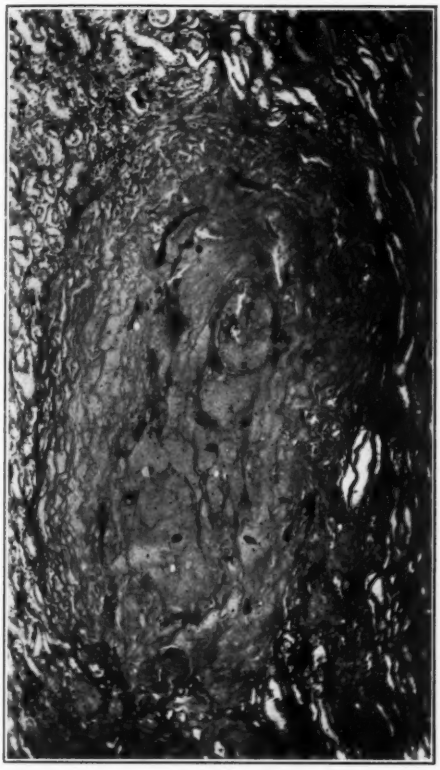

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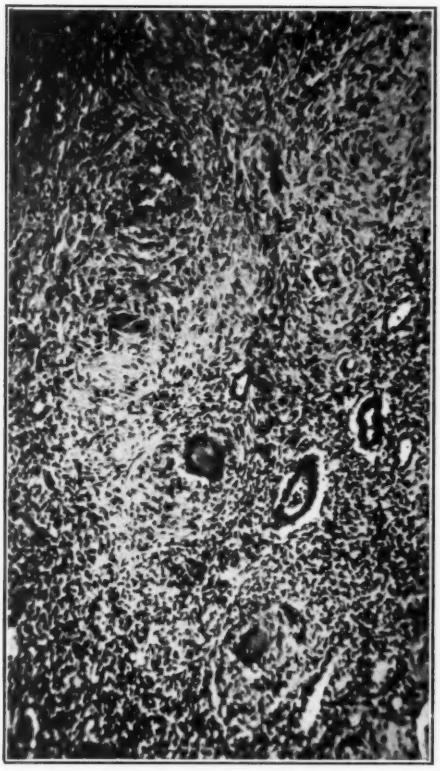

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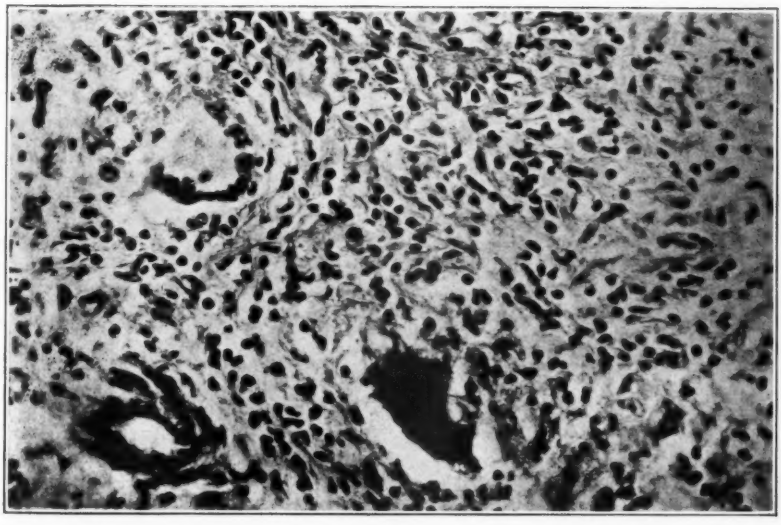

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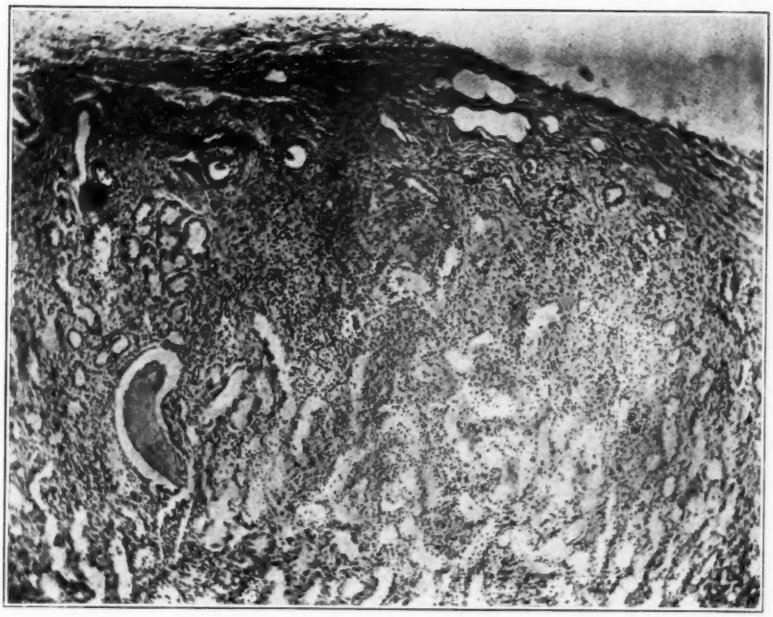

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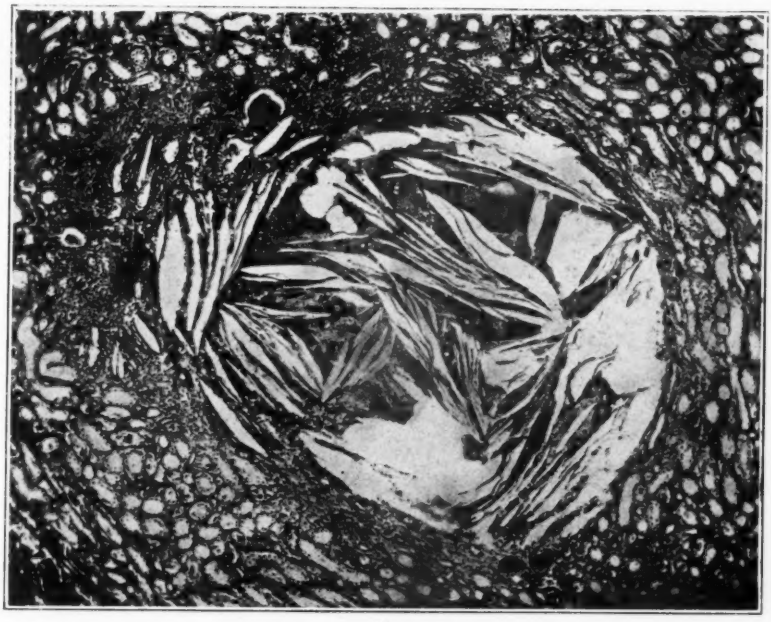

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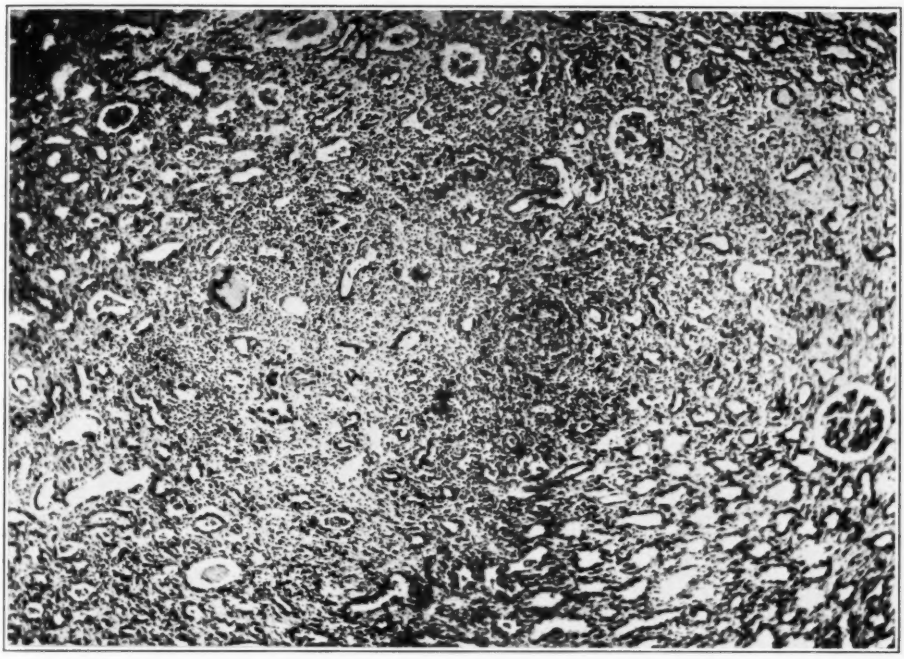

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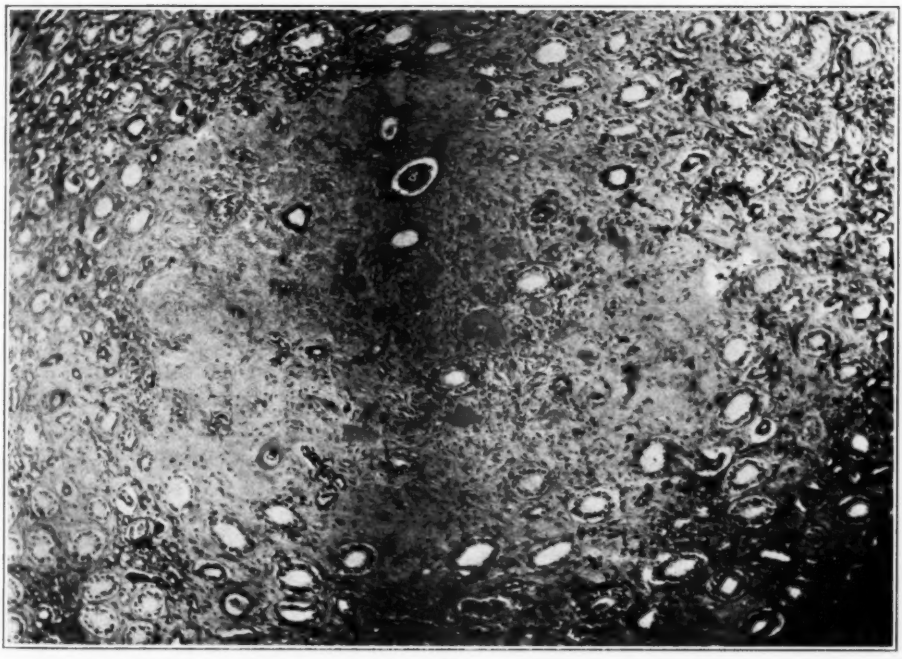

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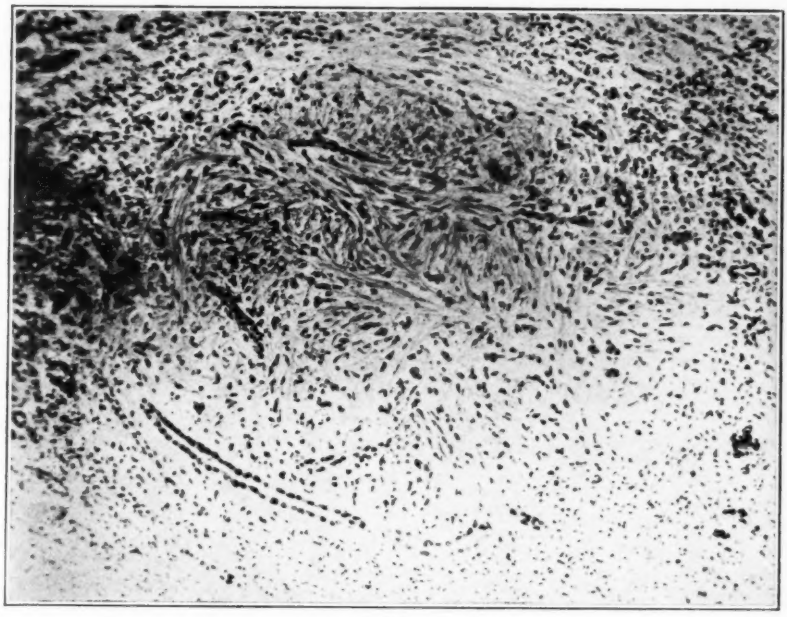

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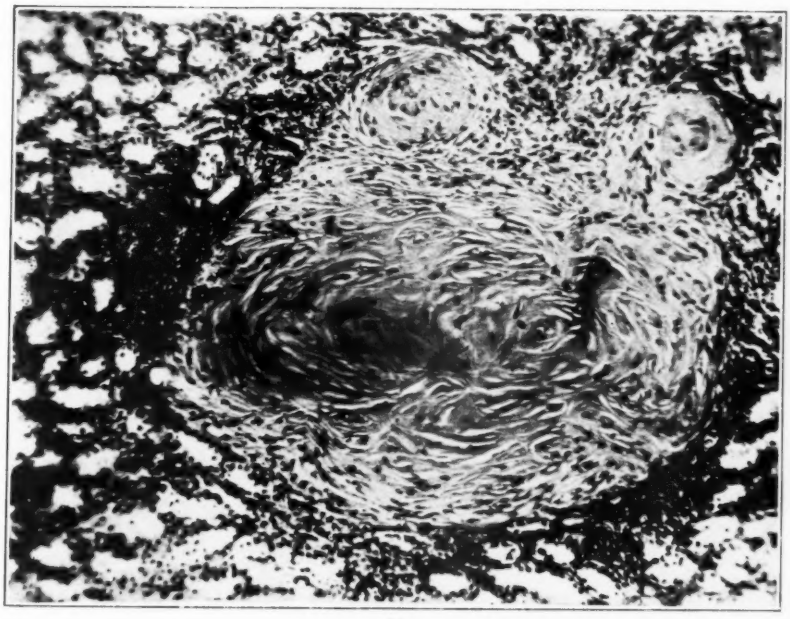

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Medlar Renal Infection in Pulmonary Tuberculosis

EXPERIMENTAL PRODUCTION OF GENERAL PERITONITIS WITH AN ANATOMIC STUDY *

BERNHARD STEINBERG, M.D.
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Three general methods are employed to produce experimental peritonitis: (1) intraperitoneal injection of microörganisms; (2) perforation of bowel; (3) ligation of the appendix. The results regarding life and the local peritoneal lesion vary with the species of animal employed.

White rats, guinea-pigs and rabbits succumb rapidly to intraperitoneal injection of fairly virulent bacteria. The peritoneum of the animals which survive for fourteen hours or longer following the intraperitoneal injection, may show a generalized hyperemia, slight deposition of fibrin and a small volume of a free gray fluid containing fibrin, polymorphonuclear leucocytes, some mononuclears and bacteria. In some instances the peritoneum shows no evidence of pathologic changes.

Benians,[1] using minute numbers of *Bacillus coli*, failed to produce death in the rabbit upon intraperitoneal injection; but when the microörganisms were mixed with gum tragacanth, death followed.

Cats and dogs seldom succumb to intraperitoneal injection even of very virulent microörganisms (Steinberg[2]). Ligation of the appendix (Costain,[3] Lehman and Copher[4]) produces death in forty-eight hours in a majority of the animals. Those dogs that survive have a localized abscess around the appendix (Steinberg; unpublished). Perforation of bowel occasionally results in death; if the animal survives, the peritoneum may show nothing or a few local abscesses.

When gum tragacanth is added to a culture of *B. coli* and injected intraperitoneally into dogs, the animals invariably succumb (Steinberg[2]). The peritoneum of these animals shows in six hours a severe hemorrhagic serofibrinous peritonitis. Gum tragacanth does not act

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merely as a foreign body (Steinberg and Goldblatt[5]) but probably possesses other properties by virtue of being a gum.

None of these methods results in a chronic or a healing acute general peritonitis such as is commonly seen in man.

Methods of Producing Experimental Peritonitis

1. Intraperitoneal Injection of Organisms
   (*a*) alone
   (*b*) with foreign bodies
   (*c*) with gum tragacanth
2. Perforation of Intestines
   (*a*) at various levels
3. Ligation of the Appendix
   (*a*) ligation only
   (*b*) ligation with perforation
   (*c*) ligation with injection of bacterial cultures into the lumen of the appendix
4. Intraperitoneal Injection of *B. coli* and its Antiserum

Intraperitoneal injection of *B. coli* and its antiserum

Steinberg and Ecker [6] produced an antiserum in rabbits against the soluble toxic substance of *B. coli*. Rabbits injected intravenously with this serum survive an otherwise fatal intraperitoneal injection of *B. coli*. The peritoneum of the rabbits receiving the antiserum intravenously and the *B. coli* intraperitoneally seldom shows any pathologic changes.

B. coli antiserum and a twenty-four hour broth culture of *B. coli* were mixed in equal volumes and incubated for one hour at 37 C. At the end of the hour, cultures of the mixture gave a profuse growth of *B. coli*.

Seven rabbits were used. Each animal received 30 cc. of the antiserum-culture mixture (15 cc. of antiserum and 15 cc. of culture) intraperitoneally.

Animals were killed at intervals of 14 hours, 18 hours, 1 day, 2 days, 3 days, 4 days and 11 days.

In fourteen hours a fibrinous exudate appeared on the diaphragm, surface of the liver, spleen and intestines. The large bowel was moderately hyperemic. Approximately 20 cc. of a grayish yellow, turbid fluid was found free in the peritoneal cavity. The fluid con-

tained fibrin, numerous polymorphonuclear leucocytes, some mononuclears and colon bacilli.

Microscopically, the peritoneal surfaces presented a layer of fibrin within which were enmeshed bacterial masses, mononuclear cells and leucocytes. Although the free peritoneal exudate contained many polymorphonuclears, the fixed tissue reaction was predominantly mononuclear in type.

In 18 hours, 1 day and 2 days, the fibrin increased in amount, binding by easily broken adhesions the omentum to the liver, loops of bowel to omentum and to each other (see Fig. 1).

In three days the diaphragmatic lymphatics were filled with bacterial masses, mononuclear cells and leucocytes which were also found between muscle bundles.

In four days the infection extended through the diaphragm and a fibrinous exudate appeared on its pleural surface (Fig. 2). The upper surface of the liver was adherent to the diaphragm by easily broken fibrinous adhesions. When pulled away, the surface of the liver was covered by a thick layer of fibrin. Microscopically, the fixed tissue exudate consisted predominantly of mononuclear cells, some of which (2 to 4 per field $\times$ 500) had become multinucleated (Fig. 3). The peritoneal and omental lymph nodes were hyperplastic. The omentum showed an infiltration of small mononuclear cells (Fig. 4). The free peritoneal fluid contained fibrin, many polymorphonuclear leucocytes and some mononuclears and but an occasional colon bacillus.

In eleven days the peritoneal cavity contained 100 cc. of a thick, gray fluid containing a great amount of fibrin, 52 per cent polymorphonuclears, 44 per cent small and large mononuclears and 4 per cent large mononuclear cells with vacuolated cytoplasm. No *B. coli* were seen and none was obtained on culture. Elevated grayish white patches several millimeters in diameter, some of them fused, were present on the diaphragm, omentum, liver, intestines and parietal peritoneum. Fibrous adhesions joined the omentum to the liver, stomach and loops of bowel (Fig. 5). Microscopically, the grayish white patches represented small abscesses surrounded by a thin layer of mononuclear cells, young fibroblasts and connective tissue (Figs. 6 and 7). At this period, fibrosis was established.

INTRAVENOUS IMMUNIZATION AND INTRAPERITONEAL INJECTION OF *B. COLI*

Benians[1] injected rabbits intravenously with small numbers of *B. coli*. Subsequent intraperitoneal injection of a fatal dose of *B. coli* produced no ill effects.

Three rabbits were each given intravenously 1 cc. salt solution containing one twentieth of a twenty-four hour agar slant growth of colon bacilli. This was repeated at the end of one week. Ten days after the last intravenous injection, 15 cc. of a twenty-four hour broth culture of *B. coli* (which proved fatal for a control rabbit in eight hours) was injected intraperitoneally into each of the rabbits. One rabbit was killed twenty-four hours later, the second animal forty-eight hours after the intraperitoneal injection and the third rabbit seventy-two hours after the injection.

None of these rabbits revealed any pathologic changes of the peritoneum.

INTRAPERITONEAL INJECTIONS OF (*a*) *B. COLI* ANTISERUM, (*b*) *B. COLI*, (*c*) *B. COLI* AND NORMAL SERUM

Three rabbits were injected with the antiserum against the soluble toxic substances of *B. coli*. Each rabbit received intraperitoneally 15 cc. of the serum. The animals were killed at intervals of 24, 48 and 72 hours. None of the three animals revealed any pathologic changes of the peritoneum.

Three rabbits were injected intraperitoneally with a twenty-four hour broth culture of *B. coli*. Each animal received 15 cc. of the culture. The three animals died in from eight to fourteen hours. The peritoneum of two animals showed a little free cloudy red exudate and slight hyperemia over the large bowel. The third rabbit, which survived for fourteen hours, had in addition a little fibrinous exudate on the diaphragm, liver and intestines.

Three rabbits were injected intraperitoneally with a mixture of normal rabbit serum and a twenty-four hour broth culture of *B. coli*, which was incubated for one hour at 37 C. Each animal received 30 cc. (15 cc. of normal serum and 15 cc. of *B. coli* culture). Two rabbits died eight hours later. One of these presented a slight hyperemia over the large bowel and a gaseous distention of the intestines; the other animal had a slight fibrinous exudate on the

peritoneal surface. The third rabbit survived and was killed on the fourth day. There was no free exudate in the peritoneal cavity. Cultures were negative for colon bacilli. The peritoneal surfaces were covered by small patches of fibrin. The infection extended through the diaphragm into the pleural cavities.

Summary

1. A method of producing acute and healing peritonitis is presented. This is accomplished by intraperitoneal injection of a twenty-four hour culture of colon bacilli together with an antiserum against colon bacilli.

2. Previous active immunization, and passive immunization induced by intravenous administration of antiserum, prevent the formation of peritonitis when *B. coli* cultures are injected intraperitoneally.

3. In the peritonitis produced under conditions of the experiment, the predominant type of cell differs in the free exudate within the peritoneal cavity from that in the fixed tissues. The polymorphonuclear leucocyte is the predominating cell in the free exudate and the mononuclear leucocyte predominates in the fixed tissues.

I desire to express my thanks to Professor H. T. Karsner for his many and helpful criticisms and his encouragement.

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DESCRIPTION OF PLATES

PLATE 86

FIG. 1. Eighteen hour peritonitis. Fibrin on diaphragm; omentum adherent by fibrinous adhesions to ascending colon and stomach. Fibrinous exudate on parietal peritoneum.

FIG. 2. Pleural surface of diaphragm in four day peritonitis. Note the grayish white fibrinous exudate.

PLATE 87

FIG. 3. Fixed tissue reaction in four day peritonitis. Note the predominant number of mononuclears and the two multinucleated cells, one in the center of the field, the other in the left lower corner. × 500.

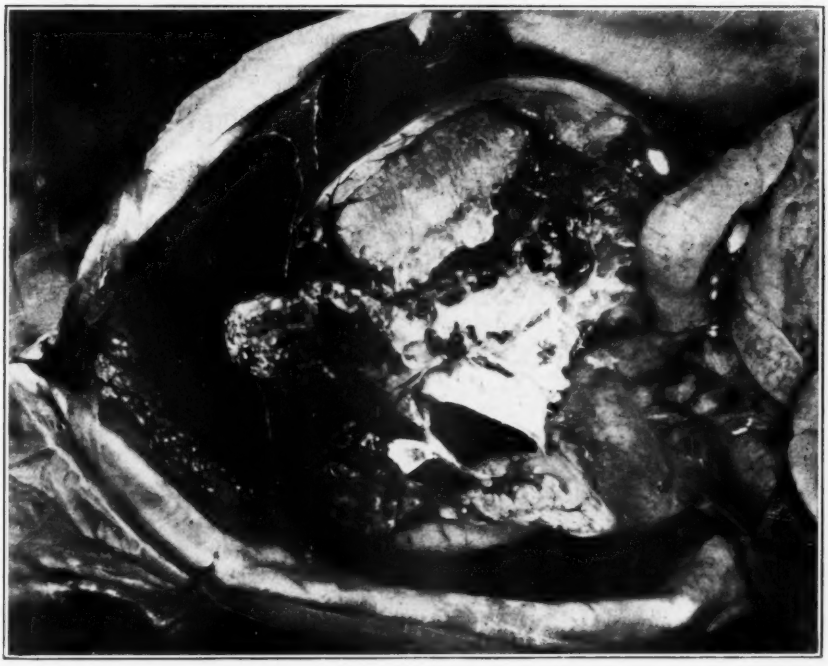
FIG. 4. Omentum and lymph node in four day peritonitis. Note the enlarged germinal center, the hyperplasia of lymphocytes and the infiltration of omentum by small mononuclears. × 100.

PLATE 88

FIG. 5. Eleven day peritonitis. Note the elevated patches on diaphragm, omentum and intestinal loop. Adhesions extend from omentum to liver, loops of bowel and stomach.

FIG. 6. Eleven day peritonitis. Note the small abscess and surrounding area. × 35.

FIG. 7. High power field of square in Fig. 6. Note the fibroblasts, connective tissue and mononuclear cells surrounding the abscess. × 500.



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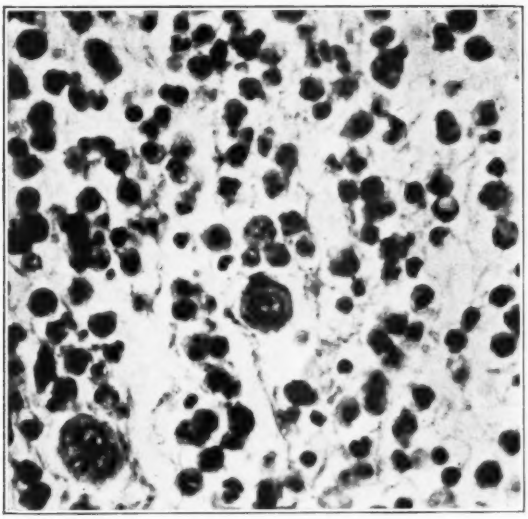
Steinberg Experimental Production of Peritonitis



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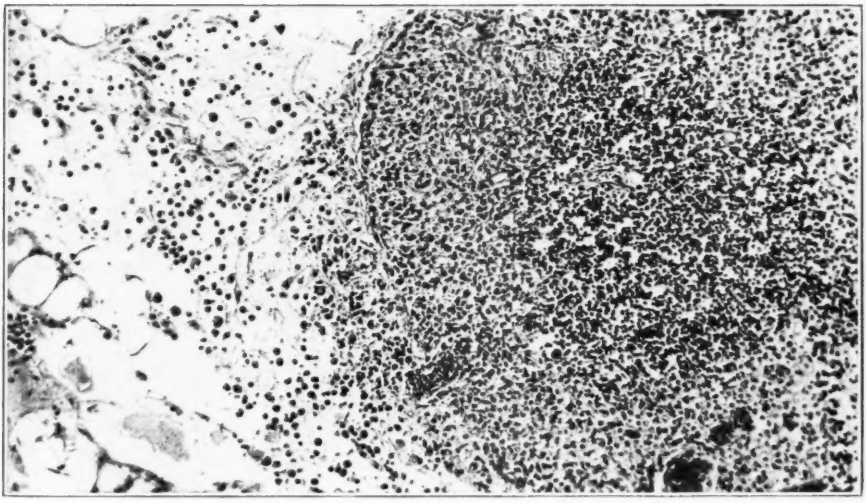

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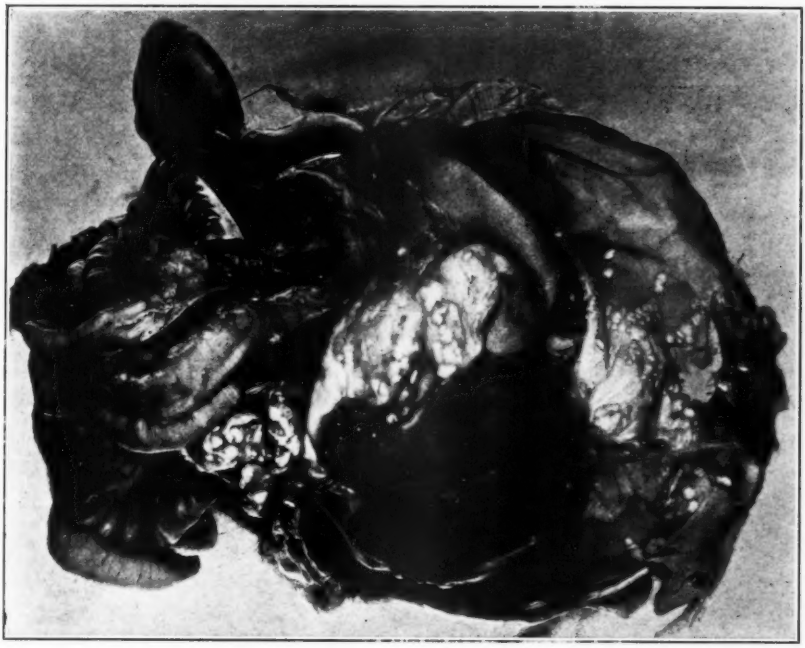

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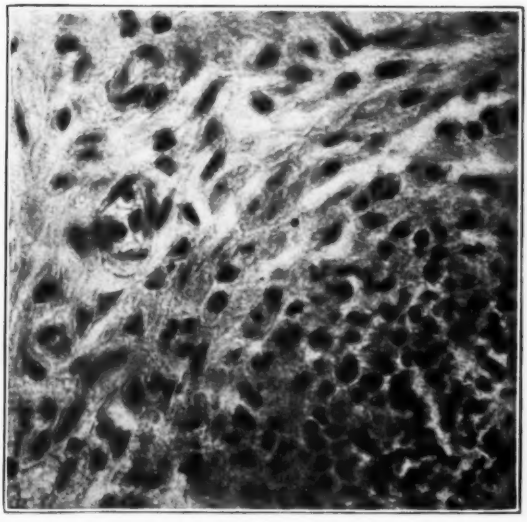

7

Steinberg Experimental Production of Peritonitis

ON DIMINISHED RESISTANCE FOLLOWING SUPRARENALECTOMY IN THE RAT AND THE PROTECTION AFFORDED BY AUTOPLASTIC TRANSPLANTS *

HENRY L. JAFFE, M.D.

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The resistance of suprarenalectomized animals to drugs and toxins has been studied by many investigators during the last thirty years. The earlier literature, reviewed and summarized in the recent papers of Lewis [1] and of Scott [2, 3], is unsatisfactory because of the lack of proper methods and control animals.

In rats between five and thirty days after suprarenalectomy, Lewis reported increased susceptibility to drugs, especially morphin to which these animals were from 400 to 500 times more sensitive than normal rats. Scott also reported marked diminution in the resistance of suprarenalectomized rats to morphin when tested from seven to fourteen days after operation. He found, too, that such animals succumb readily to bacterial intoxication, and showed that a dose of killed pyogenic cocci can be obtained that is invariably fatal to recently operated rats but never to controls. Jaffe and Marine [4] confirmed these results, injecting standard typhoid vaccine intraperitoneally, and showed that in a certain percentage of the animals compensation takes place in about nine weeks, when the rats withstand doses that would have proved fatal two weeks after operation. It will be shown that compensation, when it occurs, is dependent upon the hypertrophy of accessory suprarenal cortical tissue. Stewart and Rogoff,[5] Rogoff and De Necker,[6] and Rogoff and Ecker [7] state that, if tested after having fully recovered from the immediate operative effects, suprarenalectomized rats do not show increased susceptibility to toxins and drugs.

The purposes of this communication are: (1) to bring further proof that recently suprarenalectomized rats, fully recovered from the immediate operative effects, are highly susceptible to small doses of typhoid vaccine; (2) that as late as five months after operation, suprarenalectomized rats having no gross suprarenal accessory tis-

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sue are still very susceptible to vaccine; (3) that suprarenalectomized animals when compensated as regards resistance, invariably possess gross cortical accessory tissue; (4) that in the absence of gross accessories, autoplastic suprarenal transplants will protect suprarenalectomized rats against typhoid vaccine.

Methods

All rats were raised in the laboratory from tame albino stock. We previously described the care of our animals, the operative technic and the postoperative treatment.[8] Necropsies were performed on all rats and a careful search was made for suprarenal tissue. Standard typhoid vaccine containing one billion bacilli per cubic centimeter and heated to 38 C was injected intraperitoneally. This vaccine is a very satisfactory test substance because the minimum lethal dose for normal 250 gm. rats is between 20 and 30 cc., amounts much larger than those needed for testing resistance. Our results obtained from forty-six animals operated upon are as follows.

Resistance of Recently Suprarenalectomized Rats to Typhoid Vaccine

The fact that a large number of suprarenalectomized rats die spontaneously from suprarenal insufficiency during the first month must be considered in the interpretation of studies in susceptibility during this period. In a series of ninety-one rats, thirty-two died within thirty days after bilateral suprarenal removal, and approximately 85 per cent of these deaths occurred between the fourth and twelfth days, after which the critical period is past. If the injection of small doses of vaccine causes many fatalities after the twelfth day, these deaths may with reasonable certainty be attributed to the vaccine, and not to the moribund condition of the animals.

Eleven doubly suprarenalectomized rats, active and eating thirteen to sixteen days after operation, were injected with typhoid vaccine in doses ranging from 1 to 2 cc. Eight of these or 72 per cent were killed within nine hours by these doses which do not at all affect control rats. The suprarenalectomized animals became dull soon after the injection, some had tremors, coma ensued and death followed, preceded often by convulsions. These rats were killed by one-fifteenth to one-thirtieth of the minimum dose fatal to controls.

Table 1 concerns these eleven animals, ten of which gained weight during the postoperative period. No gross accessories were found at necropsy in the eight that died, and if present, they were microscopic and inadequate to afford protection. Of the three animals that survived, one was killed 129 days after suprarenalectomy by 5 cc. of vaccine; the other two survived 8 and 10 cc. 186 days after operation but when killed huge accessories were found.

Resistance to Typhoid Vaccine Long after Suprarenalectomy

A. *In rats without accessories.* It has been reported that the increased susceptibility of suprarenalectomized rats to toxins and drugs diminishes and completely disappears in time, but this is only partially true. When suprarenalectomized rats are followed for a year after operation, excluding those that die during the first month, the rest fall into two groups clinically and anatomically — those suffering from chronic suprarenal insufficiency with no gross accessories, and those biologically normal animals which invariably have large cortical accessory masses.

As late as five months after suprarenalectomy, the rats without accessories succumb to relatively small doses of vaccine, and Table 2 summarizes the data concerning twelve such animals which were injected between 42 and 129 days after suprarenalectomy and were killed by as little as 2 cc. of vaccine. These animals were lively and in good condition at the time of injection, though they showed signs of suprarenal insufficiency, particularly emaciation.

B. *In rats with accessories.* Table 3 presents data concerning eight suprarenalectomized rats that were injected between 65 and 186 days after suprarenalectomy with as much as 10 cc. of vaccine, and survived in every instance. In each animal, accessory tissue was found which on histologic examination showed typical, highly vascularized cortex.

The animals of this group quickly recovered from the immediate operative effects. After four weeks they were clinically indistinguishable from the non-operated litter and sex controls, at no time manifesting signs of suprarenal insufficiency. Unless distinction is made between groups A and B confusion on the subject of diminished resistance is inevitable.

TABLE 1

Number	Sex	Time between suprarenalectomy and injection	Gain in weight after suprarenalectomy	Weight at injection	Amount of vaccine	Results	Remarks
		in days	*in grams*	*in grams*	*in cc.*		
35	F	15	30	115	1½	Died; 7 hours	Died from suprarenal insufficiency induced by typhoid vaccine
44	M	13	38	116	1½	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
45	F	13	39	106	1	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
31	F	16	30	123	1¾	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
32	F	16	23	113	1½	Died; 5½ hours	Died from suprarenal insufficiency induced by typhoid vaccine
33	F	16	19	105	1½	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
38	F	15	0	140	1½	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
43	M	14	37	101	1½	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
34	F	15	15	100	1½	Survived	Killed 129 days after suprarenalectomy by 5 cc. of vaccine; practically no abdominal fat; no accessories
36	F	15	13	177	2	Survived	186 days after suprarenalectomy survived 8 cc. of vaccine; killed 204 days after suprarenalectomy; 5 mm. and 1 mm. accessories
39	F	15	22	185	2	Survived	186 days after suprarenalectomy survived 8 cc. of vaccine; killed 210 days after suprarenalectomy; right 6 × 4 mm. accessory

TABLE 2

Number	Sex	Time between suprarenalectomy and injection	Weight at injection	Clinical condition at the time of injection	Amount of vaccine	Results	Remarks
		in days	*in grams*		*in cc.*		
34	F	129	198	Active; very slight emaciation	5	Died; 9 hours	Practically no abdominal fat; no accessories
53	M	110	223	Active; good condition	4	Died; 9 hours	Practically no abdominal fat; no accessories
59	F	121	177	Emaciated; good condition	3½	Died; 9 hours	Practically no abdominal fat; no accessories
56	M	108	200	Markedly emaciated; chronic insufficiency	2	Died; 8 hours	No abdominal fat; no accessories
68	F	114	164	Active	3	Died; 8 hours	No abdominal fat; no accessories
82	F	109	154	Active; emaciated	3	Died; 8 hours	No abdominal fat; no accessories
129	M	61	146	Active; somewhat emaciated	2	Died; 12 hours	Almost complete absence of abdominal fat; no accessories
147	F	47	123	Active; marked emaciation	2	Died; 9 hours	Practically no abdominal fat; no accessories
154	F	43	125	Active; considerably emaciated	2	Died; 7 hours	No abdominal fat; no accessories
156	M	43	150	Active; little emaciation	3	Died; 6 hours	No abdominal fat; no accessories
157	M	43	168	Active; no emaciation	3	Killed; dying	Complete absence of abdominal fat; no accessories
155	F	42	164	Active; slight emaciation	3	Died; 6 hours	Very little abdominal fat

TABLE 3

Number	Sex	Time between suprarenalectomy and injection	Weight at injection	Clinical condition at the time of injection	Amount of vaccine	Results	Remarks
		in days	*in grams*		*in cc.*		
36	F	(1st) 133 (2nd) 186	221 221	Active; good condition Strong; no emaciation	5 8	Recovered Recovered	Killed 204 days after suprarenalectomy; left 5 mm. accessory; right 1 mm. accessory
39	F	(1st) 133 (2nd) 186	257 266	Excellent condition Excellent condition	5 10	Recovered Recovered	Killed 210 days after suprarenalectomy; right 6 × 4 mm. accessory
71	M	(1st) 107 (2nd) 167	247 292	Very good condition Active; slight emaciation	5 10	Recovered Recovered	Killed 198 days after suprarenalectomy; left 1 mm. accessory; right 3 × 2 mm. accessory
104	F	(1st) 66 (2nd) 120	174 187	Excellent condition Active; slight emaciation	4 8	Recovered Recovered	Killed 139 days after suprarenalectomy; considerable fat; right 2 × 2 mm. accessory
103	M	(1st) 66 (2nd) 120	215 291	Excellent condition Excellent condition	5 10	Recovered Recovered	Killed 139 days after suprarenalectomy; left 2 × 3 mm. accessory
106	F	(1st) 66 (2nd) 120	193 219	Excellent condition Excellent condition	4 10	Recovered Recovered	Killed 139 days after suprarenalectomy; right 5 × 4 mm. accessory
105	F	66	189	Excellent condition	4	Recovered	Killed 139 days after suprarenalectomy; left 3 × 3 mm. accessory
95	F	(1st) 68 (2nd) 120	163 180	Strong; active Not emaciated	4 8	Recovered Recovered	Killed 138 days after suprarenalectomy; right 6 × 4 mm. accessory

TABLE 4

Number	Sex	Time between autoplastic transplantation and injection	Weight at injection	Clinical condition at time of injection	Amount of vaccine	Results	Remarks
		in days	*in grams*		*in cc.*		
47	M	(1st) 114	285	Active	4	Recovered	Killed 204 days after suprarenalectomy; 4 good sized transplants; no accessories
		(2nd) 183	358	Excellent condition	10	Recovered	
52	F	(1st) 114	209	Active; good condition	5	Recovered	Killed 202 days after suprarenalectomy; 2 large transplants; no accessories
		(2nd) 183	234	Strong and active	10	Recovered	
50	M	(1st) 123	382	Excellent condition	7	Recovered	Killed 194 days after suprarenalectomy; 3 large transplants; no accessories
		(2nd) 174	443	Excellent condition	12	Recovered	
122	F	62	144	Eating; definitely emaciated	3	Died	Died after 22 hours; 3 tiny transplants; no accessories
124	F	(1st) 62	149	Active; slightly emaciated	3	Recovered	Died within a few hours; 3 large transplants; no accessories
		(2nd) 111	191	Good condition	8	Died	
127	F	(1st) 61	154	Active; slight emaciation	3	Recovered	Killed 128 days after suprarenalectomy; 3 large transplants; no accessories
		(2nd) 110	196	Good condition	8	Recovered	
128	F	61	151	Active; slightly emaciated	3	Recovered	Killed 128 days after suprarenalectomy; 3 large transplants; no accessories
143	F	47	161	Active; good condition	5	Died	Died within a few hours; 2 large and 1 small transplant; no accessories
144	F	47	158	Active; no clinical insufficiency	4	Died	Died within a few hours; 4 large transplants; no accessories
145	M	(1st) 47	190	Very good condition	6	Recovered	Killed 117 days after suprarenalectomy; 4 large transplants; no accessories
		(2nd) 97	280	Very good condition	10	Recovered	
148	F	44	176	Active; very slight emaciation	4	Died	Died within a few hours; 2 large transplants; no accessories
149	M	(1st) 44	188	Strong; good condition	4	Recovered	Killed 117 days after suprarenalectomy; 3 large transplants; no accessories.
		(2nd) 96	284	Excellent condition	8	Recovered	
150	M	(1st) 44	210	Excellent condition	4½	Recovered	Killed 117 days after suprarenalectomy; 3 large transplants; no accessories
		(2nd) 96	279	Excellent condition	10	Recovered	
151	M	(1st) 44	196	Strong; active	4	Recovered	Killed 116 days after suprarenalectomy; 2 large transplants; no accessories
		(2nd) 96	282	Active; slight emaciation	8	Recovered	
153	M	(1st) 44	181	Strong; active	4	Recovered	Killed 114 days after suprarenalectomy; 4 large transplants; no accessories
		(2nd) 96	243	Excellent condition	8	Recovered	

Resistance of Suprarenalectomized Rats with Autotransplants to Typhoid Vaccine

When the suprarenal is transplanted autoplastically, the regenerated cortex with neither its normal blood nor nerve supply, is nevertheless capable of protecting rats having no other gross suprarenal tissue in much the same way as hypertrophied accessories do. Fifteen transplanted animals are enumerated in Table 4, ten of which survived moderately large doses of vaccine. Four deaths were caused by the first injection, administered between the seventh and ninth weeks after operation. The transplants showed marked congestion and edema, and though regenerated, were as yet not sufficiently active physiologically to protect. The fifth rat was killed by the injection of 8 cc. of vaccine 191 days after transplantation, death being attributable to an overdose. The protection offered by transplants is clearly shown when Tables 2 and 4 are compared. All suprarenalectomized rats without accessories were killed by small doses of vaccine, while only 33 per cent of the transplanted rats without accessories succumbed, and these to larger doses. The transplant experiments offer further proof that resistance to vaccine is dependent upon the presence of active suprarenal cortical tissue.

Resistance of Normal Rats to Typhoid Vaccine

Young adult rats, weighing about 250 gm. withstood large amounts of vaccine. They suffered no appreciable effects from 3 or 4 cc.; after 5 cc. they had tremors but still reacted normally to stimulation; 10 cc. induced convulsions from which the animals recovered within a few hours. Twenty to 30 cc. sometimes killed and in these instances death was not due to the injection of large amounts of fluid for the peritoneal injection of 40 to 50 cc. of saline never kills.

Discussion

In acute infection and intoxication, pathologic changes regularly occur in the suprarenals varying in intensity from congestion and edema to hemorrhage and focal necrosis. Chronic infection and intoxication in animals are accompanied by a marked hypertrophy of the cortex with alteration in the distribution and amount of the lipoids. The significance of these pathologic findings is emphasized

by experimental studies in the resistance of suprarenalectomized animals.

We injected forty-six rats and found that the removal of both suprarenals invariably leads, in the absence of gross accessory tissue, to a considerable decrease in the resistance to typhoid vaccine. This is a consequence of the loss of some function of the cortex as shown by the facts that hypertrophied accessory rests protect suprarenalectomized rats against otherwise fatal doses of vaccine, and that autoplastic cortical transplants offer protection to animals having no other suprarenal tissue.

Rogoff and his co-workers report no significant change in the tolerance to morphin or tetanus toxin following suprarenalectomy, but they tested tolerance to substances which are extremely toxic even to normal rats. Their conclusions may be disputed, particularly in regard to morphin, if Table 1 of a recent paper [6] is analyzed. Of sixty-five animals injected between eight and thirty-six days after suprarenalectomy with doses of this drug ranging from 0.1 to 0.25 mgm. per gram body weight, which is less than the minimum lethal dose for normal rats, forty-four or about 67 per cent were killed.

Suprarenalectomized rats also show a lowered resistance to natural infections. As they become emaciated many of them develop snuffles, a condition from which normal rats or those with accessories or transplants are comparatively free, despite the circumstance that these animals are kept in the same cages under the same conditions.

Little is known of the mechanism of non-specific resistance to bacterial infection and intoxication, nor of the mechanism whereby suprarenal insufficiency affects the resistance of an animal. However when more knowledge of the function of the suprarenal cortex is acquired, it will be found that this tissue plays a vital part in the regulation of the bodily responses concerned with resistance to infections, infectious predispositions and general body well-being. It is our belief that the recovery from and the clinical course of severe infections, are dependent upon the proper functioning of the suprarenal cortex.

I wish to express my appreciation to Dr. David Marine at whose suggestion this work was undertaken.

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OBSERVATION OF FORMATION OF GIANT CELLS IN TURTLE BLOOD CULTURES *

MORTIMER COHEN

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The histogenesis of the multinucleated giant cell found commonly in various inflammatory reactions and specific granulomas has long been a subject for discussion. Opinions differ both with regard to the type or types of the cells involved in giant cell formation and as to the nature of the process by which they are formed. Three theories have been promulgated to account for the multinucleated appearance: (*a*) mononuclear cells fuse to form multinucleated cells; (*b*) the nucleus of a mononuclear cell divides and the cell increases in size without the division of the cell itself; (*c*) giant cells are not true cells but are agglomerations of cells which adhere to each other and retain their individuality, or are merely agglomerations of cells included in necrotic masses.

Each one of these theories has had supporters. The earlier work was done on fixed tissues. Mallory [1] in 1911 stated that foreign body giant cells formed by fusion, contrasting them with true tumor giant cells which he said formed by multiple mitosis. Forbes [2] in 1909 in experimental work on the development of foreign body giant cells in rabbits injected with agar remarked that the endothelial cells coalesced to form giant cells. Duval and White,[3] discussing the histology of glanders, took the opposite view. They concluded that the giant cells in their lesions formed by division of the nucleus of the endothelial cell and not by fusion.

Since it has been possible to study living cells by tissue culture methods, Lambert [4] in 1912 reported the formation of giant cells *in vitro* using chick embryo spleen. He noted the fusion of cells resulting in the formation of a thick giant cell. In 1921 Lewis and Webster [5] reported the formation of giant cells in cultures from human lymph nodes. They were led to believe that the giant cells arose from large wandering cells by amitosis. They observed one case of amitosis but no signs of mitosis. Further, M. R. Lewis and

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W. H. Lewis[6] in work done with hanging drop cultures asserted definitely that giant cells (of the Langhans type) did not arise by fusion. They were of the opinion that the cells arose by division of the nuclei without division of the cytoplasm. Since then, however, Lewis and Brüda[7] cultivated tissue from a white blood cell tumor of the rat and they stated definitely that they observed fusion of epithelioid types of cells to form giant cells.

Medlar[8] recently advanced the opinion that the giant cells were either agglomerations of adherent cells or collections of cells included in necrotic masses. A similar interpretation was suggested as early as 1885 by Baumgarten and Weigert who believed the giant cells were necrobiotic structures from the beginning (Quoted by Hektoen[9]). Hektoen[9] reviewed the literature in 1898 and while he remarked that he was not prepared to give an opinion as to the mode of formation of the giant cells, he did note that the cells, under certain conditions, separated into small living cells, and for that reason, believed they were living structures.

TECHNICAL METHODS

Turtle blood was used because the experiments could be carried on at room temperature. The most commonly used preparation was the simple hanging drop of blood prepared similarly to the method described by Lewis.[10] Blood was removed from the heart by means of a syringe and a fair sized drop was placed in the center of a clean cover glass. The cover glass was then inverted and sealed over a hollow ground slide with a vaseline-paraffin mixture. A similar preparation was made after the turtle had been injected with India ink. Five drops of India ink (Higgins) were added to 10 cc. of normal salt solution, the suspension was filtered twice and 0.5 cc. was injected directly into the circulation through the heart. Preparations were made one hour after injection. A third type of preparation was made using a modified Locke-Lewis solution. A drop of this solution was placed upon the cover glass and then a drop of blood was added. In one series of experiments the Locke-Lewis solution was added on the fifth day of incubation to plain blood preparations. A fourth type of preparation was made by Sabin's[11] method using both neutral red and Janus green. These preparations, while not hanging drop preparations, were used primarily for detailed morphologic study.

EARLY CULTURES

The drop after several hours shows a clear peripheral zone with the blood cells collected in the center. At the periphery of the cell area the white cells have gathered and the entire mass is clotted. Usually, within two days, wandering cells are seen advancing into the clear peripheral zone. These cells are large, irregular in shape, actively motile and have longer and shorter protoplasmic projections. The latter vary continuously producing changes in the shape and position of the cell. The cytoplasm is finely granular and contains rod-shaped mitochondrial granules. The granules occupy almost all of the cytoplasm in the cells of earlier preparations. The nucleus is a bland ovoid body which occupies no constant position in the cell; in fact, it changes its position with the movements of the cell. In the India ink preparation, black granules are present in most of these cells usually in the neighbourhood of the nucleus. These cells seem generally to migrate toward the periphery of the clear zone. In many of the cultures on the fourth day, large irregular multinucleated giant cells are seen at the very edge of the drop, clinging quite closely to the cover slide. They are continuously altering their shape. These cells contain from four to twelve large clear nuclei arranged irregularly throughout the cytoplasm. Nucleoli are present in most of the nuclei. Granules of varying sizes, mitochondria and vacuoles are seen in the cytoplasm. Often when red cells are near (and they are always undergoing disintegration when in the neighborhood of the giant cells), they are phagocytized and found lying within the cytoplasm of the giant cell. When India ink is used in the preparations, it is found distributed irregularly in small masses in the cytoplasm.

FUSION

The fusion is observed at the height of activity which occurs most commonly on the fourth day, although in some preparations great activity persists until the eighth or ninth day, and in one culture until the twenty-fifth day. Multinucleated giant cells are seen at the edge of the drop undergoing active changes. These cells send out projecting cytoplasm in their activity and seem to attract the surrounding cells. Large irregular wandering cells in the neighborhood also produce protoplasmic processes, as they advance toward the giant cell. When a protoplasmic projection of a wandering cell

comes in contact with the protoplasm of the giant cell which is usually also projected at that point, the protoplasmic processes widen, become one, and the contents of the wandering cell flow into the giant cell, leaving no evidence whatever of the wandering cell. The giant cell, observed for hours at a time after such a phenomenon, carries on its activity as before uninfluenced save for its increase in size. Often two mononucleated wandering cells fuse in the same fashion as they advance toward the giant cell. The new binucleated cell then assumes activity as an independent giant cell or subsequently fuses with another giant cell.

There are instances in which quiescent spherical mononuclear cells, from one to two times the size of red blood corpuscles, are seen to become motile and to fuse with wandering cells or with giant cells. The steps in this transformation are definite and fairly uniform. When first observed the cytoplasm of the spherical cells is undifferentiated and the nucleus is obscured. Suddenly the cell develops an exaggerated Brownian movement, sends out pseudopodia, develops definite rod-shaped mitochondria, discloses its nucleus and begins active ameboid motion, spreading itself so that it appears identical with other wandering cells. This cell then appears to be attracted to another wandering cell and fuses with it, or advances toward a giant cell and fuses with it. Often after several moments of active movement without fusion this cell resumes its spherical undifferentiated form and becomes quiescent. Some of the cells undergo transformation two or three times and then finally fuse with another wandering or giant cell.

FRAGMENTATION

The giant cells on observation over days do not increase in size beyond a certain point. They continue to fuse with other cells, but at intervals parts break from the main cell and move away as irregular wandering cells. On rare occasions they assume a spherical quiescent appearance. Occasionally the wandering cells return and fuse with the main cell body. At times the giant cells in their activity separate into two or three multinucleated parts. In instances they fuse again, at other times they wander away and remain as individual independent giant cells.

After several days of such activity the cells become still, develop vacuoles of varying sizes, finally break up and disappear. The

time when this disintegration occurs is never constant. Some cells break up on the twelfth day while one preparation exhibited excellent cell activity on the twenty-sixth day.

Discussion

There can be no doubt as to the formation of multinucleated giant cells by fusion in our experiments. We do not wish to indicate that such cells may not also be formed by amitosis of the nucleus with division of the cells, but in hours of constant observation we failed to detect such a process. The activity within the dense portion of the drop was never observed in the living state; however, stained preparations gave no evidence that nuclear division occurred.

In our work we have confined ourselves to observations on the development of multinucleated cells from mononuclear white cells in cultures of turtle blood *in vitro*, with the single purpose of determining whether or not such mononuclear cells may fuse to form multinucleated cells. We made no attempt to determine the origin of the mononuclear white cells under observation.

The fact that whole multinucleated cells were seen to migrate as entities and that no protoplasmic divisions were perceptible either during cultural stages or after fixation and staining, practically precludes the possibility of the multinucleated masses being agglomerations of separate cells.

Difficulties in comparing mammalian and reptilian blood were encountered both with films fixed and stained, and films prepared according to Sabin's [11] technic. The type cell entering the reaction was not identified as to its origin. We were content in recognizing it as a mononuclear wandering cell with phagocytic properties, evidenced by the ingestion of India ink particles.

The India ink preparations were not different from the simple preparations save for the presence of the black particles in the wandering cells and in the giant cells.

The results of the experiments with Locke-Lewis solution were not as satisfactory. A few small multinucleated giant cells were formed in some of the preparations, but the activity was retarded.

Conclusions

1. Only large mononuclear wandering cells were seen to enter into the formation of giant cells.

2. Multinucleated giant cells were observed to form by fusion in hanging drop cultures of turtle blood.

3. Quiescent non-granular mononuclear types of cells were observed to take on ameboid activity, to show mitochondrial granules and to participate in fusion phenomena.

4. The multinucleated giant cells behaved like true cells, showed no partitions and were not merely coherent masses of single cells.

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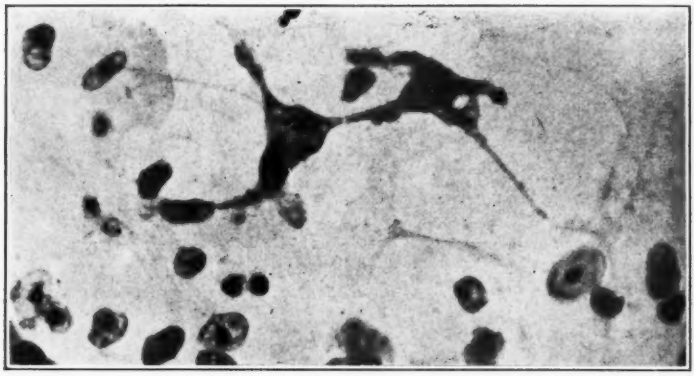
DESCRIPTION OF PLATES

Plate 89

Fig. 1. Giant cell with two nuclei showing long protoplasmic processes; elongated wandering cell in neighborhood advancing toward large cell.
Fig. 2. Beginning fusion of two large wandering cells.
Fig. 3. Giant cell from four-day culture.

Plate 90

Fig. 4. Camera lucida sketch of giant cell in eight-day culture; note wandering cell approaching giant cell.
Fig. 5. Same cell nineteen hours later; spherical forms shown, one just having taken on ameboid activity. Giant cells beginning to break.
Fig. 6. Same cell one hour later; the parts breaking further.
Fig. 7. Giant cell activity on twenty-fifth day.



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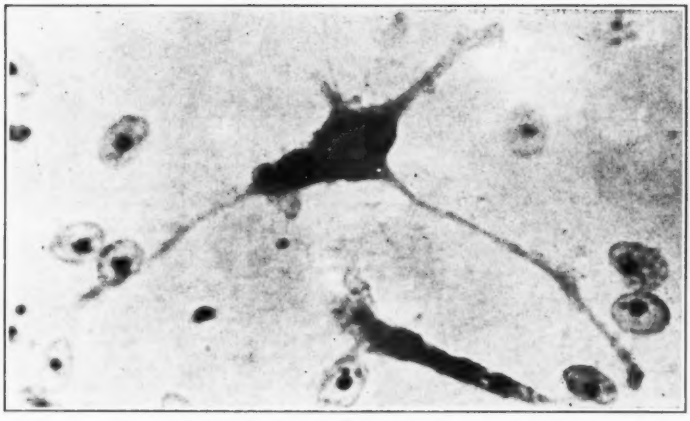

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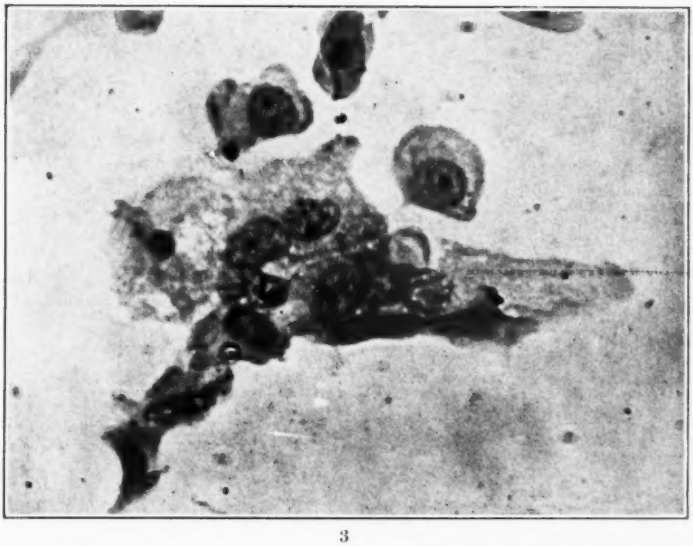

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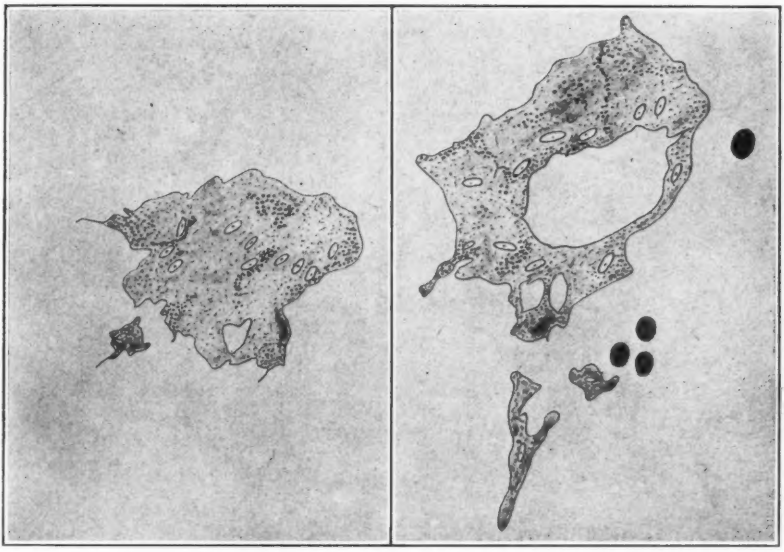

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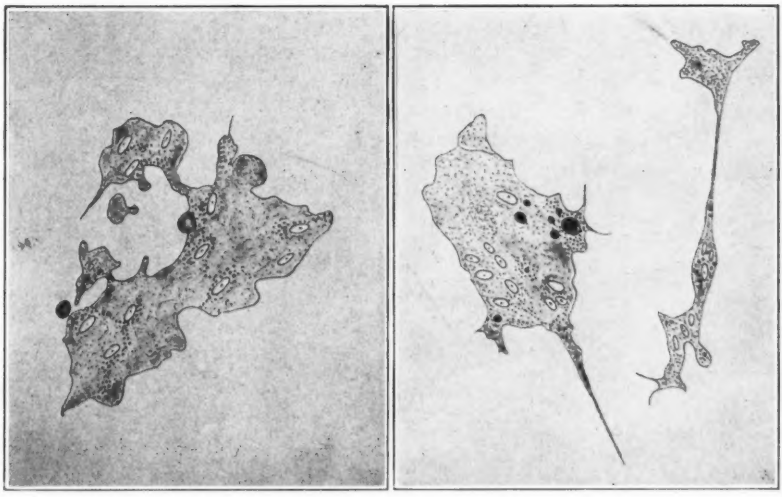

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Cohen Giant Cells in Turtle Blood Cultures

SCIENTIFIC PROCEEDINGS OF THE TWENTY-SIXTH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ALBANY, NEW YORK

APRIL 2 AND 3, 1926

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

SCIENTIFIC PROCEEDINGS

A Bacteriologic Study in Cases of Postoperative Thrombosis and Embolism. E. C. Rosenow, Rochester, Minn.

Abstract. Partial tension cultures have been made in five cases of postoperative pulmonary embolism secondary to thrombosis of iliac or femoral veins and one case of portal thrombosis. The thrombus and embolus were removed in as sterile a manner as possible, washed repeatedly in large amounts of sodium chloride solution, or the surface was seared with a searing blade. Emulsions in broth or sodium chloride solution made by grinding small pieces of the thrombus in a sterile mortar with sand, were inoculated into tall tubes of melted glucose brain agar, glucose brain broth and other mediums. Similar cultures were made from the blood and from the pipettings from the spleen, liver and kidneys. Necropsy in all of these cases was performed within twelve hours after death. A diplococcus similar to the one obtained twelve years ago in similar cases in Chicago was isolated from the embolus and thrombus in each of the five cases of pulmonary emoblism, and from the thrombus in the case of portal thrombosis. A staphylococcus was also obtained from emulsions in two cases. The number of colonies of the diplococci was not large, never more than 500 per gram of thrombus. The colonies could be seen growing on the edges of the pieces planted into the soft glucose brain agar. Primary aerobic cultures on blood agar were always sterile but subcultures grew readily on aerobic blood agar plates. The blood from the heart, liver, spleen and kidneys was sterile in four cases. The diplococcus was obtained from the blood in one instance only. In one case of sudden death from pulmonary embolism following amputation of the breast for ulcerating carcinoma and in which marked cellulitis with septic fever occurred, a hemolytic streptococcus was isolated from the wound and blood in pure culture and from the pulmonary embolus and iliac thrombus in mixture with the diplococcus. In the case of portal thrombosis associated with peritonitis following gastrectomy for carcinoma of stomach, *B. coli* and *B. welchii* were found in addition to the diplococcus. Cultures from the blood yielded *B. coli*. The organism isolated from the thrombus was much alike in the different cases. It is a gram-positive diplococcus; its size, shape and grouping resembles the pneumococcus closely but it is free from a capsule and the individual cocci are more nearly round. It produces small dry nonadherent colonies on horse blood agar plates, which are usually surrounded by a green or brownish green zone of partial hemolysis. The diplococcus is of low general virulence, it does not grow well in citrated blood *in vitro*, it shortens the coagulation time of the blood markedly, and it tends to incite thrombosis in animals following intravenous and other injections. It has been demonstrated microscopically in the thrombi produced

experimentally and in the thrombus of pulmonary embolus of all but two of twenty-five patients that died of postoperative pulmonary embolism.

Two of the strains fermented inulin and salicin; one of these also fermented mannite; the rest did not ferment these sugars. All strains fermented glucose, lactose and raffinose.

(No discussion.)

FURTHER OBSERVATIONS ON THE STREPTOCOCCUS TOXINS AND THEIR ANTITOXINS AND THE STANDARDIZATION OF THERAPEUTIC SERUMS. Mary B. Kirkbride and (by invitation) Mary W. Wheeler, Albany.

Abstract. Toxicity tests on goats indicated no fundamental qualitative or quantitative differences in the toxins of hemolytic streptococci from scarlet fever or from other infections; toxicity tests on human subjects were apparently not as dependable, as the same toxins induced variable reactions in different individuals.

As a result of titration tests on goats with eighty serums including trial bleedings, confirmed with tests of sixteen serums on human subjects, a method for standardizing scarlet fever antistreptococcus serums on goats has been developed. This depends upon comparative tests of the test serums with a standard serum against a dose of a standard toxin, made at the same time, on the same animal under carefully controlled conditions.

(Discussion by Dr. W. H. Park, New York.) I am just wondering whether the observations of Miss Kirkbride and Miss Wheeler are correct in that goats would show differences between the toxins of different strains when human beings might not be so well used. It would seem to me that the goat could not be used for that purpose as well as human beings. There is no question that in the persons who showed marked difference in the reactions to the Dochez and the Dick toxin that there was a difference between the component toxins of those two strains, while on goats there would apparently be no difference. I think it would be like testing for similarity of agglutinins and not using the absorption method. With the ordinary agglutination method many different cultures would react alike where with the absorption method they would differ. The human being therefore would be a more accurate and real test for differences than the goat.

(Miss M. W. Wheeler, Albany, closing.) In answer to Dr. Park's question — it might be possible to distinguish certain qualitative differences in the toxins of different scarlet fever streptococci more readily by the human test than by the goat test. The human test, however, apparently cannot be relied upon for differentiating the specific scarlet fever streptococci from other hemolytic streptococci, since we have found that individuals frequently vary in their reactions to toxins of different strains of streptococci isolated from typical cases of scarlet fever.

THE PREPARATION AND CONCENTRATION OF SCARLET FEVER ANTITOXIN AND ITS CLINICAL APPLICATIONS. John F. Anderson and (by invitation) George F. Leonard, New Brunswick, N. J.

Abstract. This paper reports the results of the immunization of eighty-four horses for the preparation of streptococcus scarlet fever antitoxin prepared according to the methods described and developed by the Dicks. The horses were immunized chiefly by the injection of either filtered broth cultures of scarlet fever streptococci or by the injection of fresh cultures of scarlet fever strepto-

cocci which had been passed through a Sharples centrifuge. The horses received toxin testing as high as 100,000 skin test doses per cc. Five different strains of scarlet fever streptococci were used in connection with the immunization of the horses. The antitoxin was concentrated by the Banzhaf process.

A report was made of 130 severe and moderately severe scarlet fever cases in three institutions in three different states which were treated with this antitoxin, as compared with the results in eighty-four cases in the same institution who received no antitoxin. Among the 130 cases, some of which were severe with marked toxemia, there were no deaths, and only 4.6 per cent developed complications, none of a severe character or requiring surgical intervention. Of the eighty-four mild cases, without antitoxin, there were no deaths, but 28.6 per cent had complications and sequelae of varying degrees of severity. Some of the complications were of a grave character, requiring surgical intervention, and consisted of nephritis of a serious grade, otitis media, mastoiditis and suppurating cervical adenitis.

The amount of antitoxin as given in the three institutions is shown in the following table:

Dosage

Hospital	Maximum	Minimum	Average
A	520,000 neutralizing doses	80,000	221,750
B	300,000 " "	75,000	137,850
C	600,000 " "	200,000	256,800

The results as reported are summarized as follows:

Specific scarlet fever antitoxin may be prepared by the immunization of horses with filtered toxin.

Such antitoxin is specific against scarlet fever occurring in widely separated sections of the United States.

A properly prepared and standardized antitoxin is effective as a prophylactic when used in adequate doses.

When used for passive immunization, it should be given in not less than one half of the average therapeutic dose.

A properly prepared and standardized scarlet fever antitoxin is effective in the treatment of scarlet fever, saving life and reducing the severity and frequency of complications.

(Discussion by Dr. W. H. Park, New York.) It happens that in our work we have used only a very few horses but one-half of these horses have been on the Dochez method and one half on the so-called Dick method. The most potent horse was on the Dick method but one almost as good was on the Dochez method and the fact that we have been using the serum made by both methods with excellent results, seems to me to show that either method will give highly potent and useful serum. Apparently one depends rather more on a suitable horse than on the method. As far as the annoyance goes, there is very little annoyance if you have the scarlet fever horses in separate stables. There is very little trouble from the abscesses. They are small and in a week or so heal up entirely.

There is another point I want to discuss and that is the question as to whether we should inject the serum to prevent scarlet fever in those who have been exposed. I agree with Dr. Anderson that practically every case will be protected for ten days or two weeks. On the other hand, we have the difficulty that contagion will probably persist longer than that in any institution or a family and

you will have to repeat the dose. Unquestionably you get a greater serum reaction from the present refined serum than from the refined diphtheria antitoxin, and there is still a greater difference if unrefined serum is used. This is probably due to the fact that there has been no time to age the serum. Scarlet fever is not a very contagious disease. In New York City about one in ten of children exposed in families will develop scarlet fever. I prefer, therefore, to do an immediate Dick test and then if the child or person develops a sore throat or the slightest clinical evidence of scarlet fever, then give a therapeutic dose of the serum. I have had experience in certain institutions and families where we have used this method and they have had very little reaction. So far, in over 100 exposed cases so treated there has only been one case of scarlet fever develop.

(Discussion by Miss M. B. Kirkbride, Albany.) In connection with the standardization and the production of the antitoxin, although we have had very few horses, we have had a few on the Dick method and a few on the Dochez method. Our highest titred serums, however, have been obtained with a combination of the two methods. One cubic centimeter of serum neutralizes, I think, from 20,000 to 30,000 skin test doses of toxin.

(Discussion by Dr. E. C. Rosenow, Rochester, Minn.) The results following administration of antitoxin in two cases of scarlet fever which I have seen will be of interest. Both occurred in children of one family. The fever and rash disappeared promptly following administration of the antitoxin on the second and third day, respectively. Six days later both patients developed nausea and vomiting, diarrhoea, severe gastro-enteritis, high fever, marked tenderness of abdomen, and one a pelvic abscess due to scarlatinal hemolytic streptococci. The development of the abscess was interpreted by Dr. Helmholz as secondary to enlarged retroperitoneal lymph glands because the antitoxin was given deep into the gluteal muscles. Whether this interpretation is correct or not is, of course, problematic, but in the light of these findings I would warn against the giving of scarlatinal antitoxin intramuscularly where the deep lymphatics of the peritoneal cavity may become involved.

(Discussion by Dr. James Ewing, New York.) I recently saw a child with progressive, general enlargement of the lymph nodes, severe anemia and a blood picture of aleukemic leukemia. The parents were convinced that the disease had resulted from a preventive dose of scarlatinal antitoxin. The history was consistent with this interpretation. I have heard of one other case of this same type.

While I am very skeptical about the relation between the antitoxin and the progressive anemia in these cases, both of which were fatal, I think such observations should be recorded, since, if they accumulate in sufficient numbers, they may possibly be of significance.

(Dr. J. F. Anderson, New Brunswick, N. J., closing.) In answer to Dr. Park's comments I would state that I have never advocated the routine use of antitoxin in diphtheria or scarlet fever as a preventive measure. I think it is an unsound and unscientific way of dealing with the situation. Nevertheless, there are many doctors who still use diphtheria antitoxin as a routine measure in the case of exposure of individuals to diphtheria and there are certainly a very large number who are doing the same thing with the scarlet fever antitoxin. As a matter of fact I think there is twice as much of the preventive dose of the scarlet fever antitoxin distributed as there is therapeutic. I agree with Dr. Park that what should be done in such cases is very close observation of the child and at the first evidence of infection a full sized dose of antitoxin be given.

If, however, the physician or family insist upon the administration of antitoxin as a prophylactic measure the proper procedure is certainly to begin immunizing with the toxin two weeks later in order to bring about active immunity.

In regard to Miss Kirkbride's comments — we have never used the Dochez method but we have been using the method suggested by Zinsser and we believe that it has many points of advantage. That is the method in which you use oxalated blood inoculated with the streptococcus and grown overnight. Immediately before injection add a proper amount of calcium chloride to bring about clotting after injection into the animal. We have five different groups of horses, not less than five in any group which are under different methods of immunization. We have a group given filtered toxin, another group is given filtered toxin plus injections of live cultures. Another group is given the Zinsser method alone. Another group Zinsser method plus the subcutaneous injections of the filtered toxin. In the course of the next few months we will have something that will at least indicate to us whether any of these various methods offer much over another.

STANDARDIZATION OF SCARLET FEVER ANTITOXIN. William H. Park, New York.

Abstract. There are difficulties met with in carrying out the Dick method of standardizing scarlet fever antitoxin. The subjects must be human beings who while strongly positive to the Dick test are not sensitive to the horse serum containing the antitoxin to be tested.

Some persons who show the same degree of reaction to the Dick test as others do not respond equally. Unless a great many tests are carried out it is impossible to test the potency of a serum within fine limits.

Certain breeds of goats have been used by Kirkbride and Wheeler with success. They respond to even smaller amounts of toxin than human beings. Some of them are sensitive to horse serum and all become so a few days after testing. The great advantage is that we are relieved from testing on human beings. The potency of a serum can be tested within broad limits. The Schultz Charlton blanching phenomenon can be used for testing the potency of a serum as brought out by Blake and Dochez. On the chart you see the results of using four serums in different dilutions. One cc. of a 1:100,000 dilution of a serum that neutralized 50,000 Dick skin test doses of toxin caused definite blanching in suitable cases. This method has its difficulties. It is necessary to have access to scarlet fever cases with suitable rashes, which will not receive serum treatment. An evenly distributed erythema on the first or second day of its appearance is the best and it must remain bright for at least eighteen hours. Even then different rashes give different potency tests. Older rashes are not so good and give lower readings. Unless a great many tests are made only a fair estimate of potency can be made.

All these methods, however, give tests of sufficient accuracy to be used by us in the treatment of cases. A number of us are now arranging to have a large amount of serum standardized so that the standard serum can be compared with the serum to be tested. This will make for more accurate results.

(No discussion.)

FACTORS INFLUENCING THE POTENCY OF SCARLATINA TOXIN. Anna W. Williams, New York.

Abstract. The toxins reported here were all prepared one year or more ago. We have observed the effect of the different factors mentioned both on the potency immediately after preparation and on the permanency of that potency.

1. Amount of peptone. Two per cent peptone (Digestive Ferments proteose or Park Davis) gives a stronger toxin than 1 per cent but the strength of the toxin drops more rapidly and approaches in time the same potency as the 1 per cent.

2. Blood. The same thing is true for blood. All bloods tried give a stronger toxin than the same lot of broth without blood, but the drop is proportionately great.

3. Reaction. With a less alkaline reaction (pH 9.4) the toxin is stronger than with a more alkaline one (pH 8.2) up to the fifth day; after that up to the tenth day the more alkaline medium gives a stronger toxin.

4. Time of growth in incubator. The longer growth (7 to 10 days) has given a more stable toxin.

5. Animal passage. Strains greatly increased in virulence by mouse passage make a much stronger toxin than the original strains but the drop in toxicity is greater. However, their final potency remains appreciably greater than that of the toxin from the original strains.

6. The drop of potency of these very strong toxins is variably rapid. In about two months they may drop 50 per cent — in another month a half more, and after that the drop is slower.

(No discussion.)

THE EFFECT OF CINCHONICS ON PNEUMOCOCCI. I. THE INFLUENCE OF QUININ UPON PROLIFERATION AND AUTOLYSIS OF PNEUMOCOCCI, WITH SPECIAL REFERENCE TO THE EFFECT OF HYDROGEN ION CONCENTRATION. Meyer Solis-Cohen, Philadelphia.

Abstract. The germicidal action of the quinin salts against pneumococci increases with increase in alkalinity. An increase in effectiveness begins to become evident at the reaction of normal blood (pH 7.4) but its maximum effect is shown in a reaction more alkaline than this.

An interesting phenomenon becomes evident when the effect of quinin in buffered broths of varying hydrogen ion concentration is studied. In broths of pH 6.3 to 6.8 of such acidity that growth of pneumococci normally does not occur, pneumococci are stimulated to growth within a certain zone of quinin dilutions (1:15,000–1:50,000 quinin hydrochloride, depending on the pH). No growth occurs in concentrations of quinin higher or lower than those in this zone.

In a study of the effect of quantity of organisms upon the pneumococcidal action of quinin, it was noted that upon diluting the seed culture a dilution was eventually attained that failed to grow in the control tube of broth but that was stimulated to growth in weak dilutions of quinin.

Especially with optochin, growth of pneumococci in very high dilutions was less favorable than in the control tube; in moderate dilutions, growth was as marked or more marked than in the control tubes; in low dilutions inhibition and death of the organisms became evident.

In the presence of quinin, autolysis of pneumococci within a period of twenty-four hours is extended to a range of hydrogen ion concentration more alkaline (between pH 5.5 and 7.6) than that in which dissolution occurs in the absence of quinin (between pH 5.5 and 6.8). This phenomenon is pronounced in a concentration of 1:4000, is still markedly evident in a concentration of 1:20,000 but becomes less evident in a concentration of 1:100,000 and practically nonexistent in a dilution as great as 1:400,000.

There is, furthermore, a tendency, particularly in the alkaline broths, for autolysis to occur at an earlier period in dilutions of quinin than in the broth control tubes.

(Discussion by Dr. C. W. Jungeblut, Albany.) I have seen in one of these charts that pneumococci which had been cultivated in the very high dilutions of optochin apparently did not grow as well as those from the more concentrated dilutions. This fact which, *a priori*, is difficult to understand, might be explained by the phenomenon which Schnabel described five years ago. He found that pneumococci after having been exposed to very high dilutions of optochin, such as one to ten or one to twenty million, would exhibit at the next passage a specifically increased sensitiveness to the drug, while those obtained from the lower concentrations became fast.

(Dr. Meyer Solis-Cohen. No reply.)

THE EFFECT OF CINCHONICS ON PNEUMOCOCCI. II. CONTRASTED BEHAVIOR OF PNEUMOCOCCI TOWARD QUININ AND OPTOCHIN IN RELATION TO DRUG FASTNESS. Meyer Solis-Cohen, Philadelphia.

Abstract. Resistance of pneumococci to optochin may be developed very quickly. Within an experimental period of twenty-four to forty-eight hours there is acquired an insusceptibility to concentrations ten and more times as great as that in which growth of the untreated culture is inhibited. In our experiments, resistance developed whether the organisms were continuously or intermittently exposed to optochin.

Various experimental methods were tried to determine whether pneumococci can become insusceptible to the germicidal action of quinin upon prolonged exposure to a salt of this alkaloid. The experimental periods ranged from ten to thirteen days. In one series of experiments, 10 per cent of the cultures eventually grew in the highest dilution in which the control culture failed to grow. In no other series could even this extent of resistance be demonstrated. Against quinin, therefore, pneumococci do not show a similar tendency to the development of resistance.

(Discussion by Dr. C. W. Jungeblut, Albany.) I would like to know whether any tests were done to determine the cultural characteristics of the fast strains, and also whether the specificity of this fastness was determined.

(Discussion by Dr. Charles Weiss.) Optochin, as you know, is a synthetic salt of quinin, also known as ethyl hydrocuprein hydrochloride. Dr. Kolmer and I worked with this as well as with a number of other quinin compounds, and it is interesting to note that there is a marked difference in the activity of synthetic and natural compounds. One of the interesting things we observed which is very striking is that the intracutaneous test which can be used to demonstrate hypersensitiveness to quinin salts (first shown by Boerner) behaves differently when using the synthetic salt and when using the natural salt. I used this skin test on Dr. Boerner, who is extremely hypersensitive to quinin,

employing a series of about fourteen different salts, beginning with quinin alkaloid all the way up to the synthetic quinin. I found that although Dr. Boerner reacted to the natural quinin salts he did not react to the synthetic quinin, optochin.

(Dr. Meyer Solis-Cohen, closing.) I do not think the tests mentioned by Dr. Weiss were made.

THE EFFECT OF INTRAVENOUS INJECTIONS OF INDIA INK IN THE IMMUNIZED ANIMAL. Claus W. Jungeblut and (by invitation) Joseph A. Berlot, Albany.

Abstract. Massive doses of India ink injected intravenously into guinea-pigs before immunization with toxin-antitoxin mixture caused a delay in the appearance of diphtheria antitoxin for about one week. Intravenous injections of India ink into guinea-pigs were followed by a transitory drop of the complement titer. Reduction tests (methylene blue and nitro-anthraquinone) showed the respiration of the cells of the liver and spleen of the same animals to be markedly impaired for the first day. India ink injected intravenously in large doses into guinea-pigs before active sensitization caused a more or less marked decrease in sensitiveness to the reinjection of the antigen, while, when injected before passive sensitization, it did not interfere with the occurrence of anaphylactic shock. The precipitin titer of blocked sensitized rabbits was somewhat lower than that of controls. The titer of anaphylactic antibodies differed only in one case.

(Discussion by Dr. F. P. Gay, New York.) This last chart is particularly interesting in connection with antibody formation. It fills a gap which must exist between the apparently diverse results of Lewis who thought that blocking, or rather tuberculosis in his case, stimulated antibody formation and those observations, including our own, which indicate that antibody formation may be prevented with trypan blue. It is merely a relative difference. A small amount of colloidal blocking substances will stimulate antibody formation, and a larger amount may prevent it entirely. I think this last experiment is a very clear representation of that point.

(Dr. C. W. Jungeblut, closing.) I agree with Dr. Gay that the discrepancies reported in the literature may be fully explained by the fact that different authors used different experimental animals, different substances, different doses and also determined the antibody titer at different times during the immunization.

STUDIES ON IMMUNITY TO TUBERCULOSIS. Henry Stuart Willis, Baltimore.

Abstract. The most acceptable view of immunity to tuberculosis is that this phenomenon is very closely related to hypersensitiveness to tuberculin. We know that hypersensitiveness as shown by the tuberculin reaction in human beings may fluctuate considerably during normal life, becoming very slight or completely absent at times and developing again at other times. The question of whether immunity to tuberculosis fluctuates in this manner is an engaging one and one the answer to which has been sought by the following experiment.

The plan of the experiment was to study tuberculin sensitiveness of the skin of guinea-pigs for a relatively long time after infection and to compare the state of allergy as shown by skin reactivity with the degree of immunity to reinfection. To this end, guinea-pigs were first inoculated with a strain of tubercle bacillus of low virulence (R1) and at trimonthly intervals, beginning one year after in-

fection, a certain number of them were given intracutaneous tests with tuberculin and were, a few days later together with normal animals, inoculated subcutaneously with an emulsion of virulent tubercle bacilli.

Normally, the reaction to tuberculin injected into the skin of hypersensitive guinea-pigs is an inflammation, characterized by redness, induration and frequently hemorrhage and necrosis, the height being reached within twenty-four to forty-eight hours of the injection. The animals in this experiment that were tested with tuberculin one year after the initial, sensitizing inoculation (R_1) were found in general to give very vigorous reactions. Animals allowed to go eighteen months before test were also found to react but with less vigor. At twenty-one months the reaction in those tested was still less marked and usually by twenty-four months no reaction could be elicited with the ordinary doses of tuberculin. Some of these latter animals, however, gave reactions with larger doses of tuberculin.

After each group of animals were tested with tuberculin they were inoculated with virulent tubercle bacilli (H_{37}). Those receiving the second inoculation, two years after the initial infection, although they had lost their skin sensitiveness, still had a high degree of immunity — as high as had those reinfected at 12, 15, 18 or 21 months after the sensitizing infection or as had animals reinfected only two or three months after initial infection.

It was interesting to note that, although the skin-sensitiveness of the animals two years after the initial infection was in abeyance and could not be elicited with the dose of tuberculin ordinarily used, yet this sensitiveness was very quickly restored after reinfection, for the animals four or five days after reinfection exhibited very marked reactions to tuberculin.

The question arises as to whether immunity and hypersensitiveness do actually coexist *at all times* and whether fluctuation in hypersensitiveness necessarily indicates fluctuation in immunity. It appears that the degree of immunity of infected animals does not decline quantitatively in proportion to the decline of allergy *as we have been accustomed to test these phenomena*. But the failure to give skin reactions is perhaps not to be considered as absolute proof of the complete absence of hypersensitiveness. Is the immune state one of delicate balance, which, though not always demonstrable to test, may be present and may very quickly be raised to a high degree? There may be an immunity of minimal stationary degree which is present so long as infection exists or so long as any hypersensitiveness exists, even in slightest degree.

(Discussion by Dr. S. A. Petroff, Saranac Lake.) I was very much interested in Dr. Willis' paper. We have been carrying on the same type of experiment for the last four or five years, using, however, dead tubercle bacilli for our sensitization and not the strain of culture used by Dr. Willis, which is RI, a culture of low virulence. We have found that skin hypersensitiveness produced in guinea-pigs with dead tubercle bacilli is not of the transitory type. A fairly good hypersensitiveness lasting for over a year can be set up in a perfectly normal animal with dead organisms.

Another thing that interested me considerably in Dr. Willis' paper was the relationship noticed between skin hypersensitiveness and immunity in his animals. As I understand from what he tells us, guinea-pigs inoculated with RI organisms become skin hypersensitive, which state may last a year or two, after which the animals become skin negative and at this stage, although not hypersensitive, a degree of resistance could be demonstrated.

Our experience has been somewhat similar to that of Dr. Willis. In a large

series of experiments we have noticed that animals treated with dead tubercle and having lost their hypersensitive state, still possess some degree of resistance.

I am inclined to believe that we may be wrong in saying skin hypersensitiveness means resistance and that in all probability it has nothing to do with the basic mechanism of immunity.

(Discussion by Dr. R. R. Mellon, Rochester, N. Y.) I would like to ask a question about the time of the development of the immunity processes in relation to the time of development of hypersensitivity. Is it definitely known whether the time of the immunity development is synchronous with the allergic development or does it come sometime later?

(Discussion by Dr. L. Dienes.) The close relation between hypersensitivity and immunity was questioned by Lowenstein on the ground of the experience with bovo-vaccination according to Behring. After vaccination it was found that the majority of the resistant cattle did not react to the subcutaneous administration of tuberculin. Also in recent experiments of Calmette on cattle, with the B. C. G. bacilli, it was found that the hypersensitiveness, determined in this case with the intracutaneous test, disappears four to twelve months after vaccination, whereas the animals tested eighteen months after the vaccination were found resistant.

In forming any conclusion from this observation great caution is necessary because we do not exactly understand what is really indicated by the skin test, and on the other hand it is known that the result of the test is influenced by the technic and by the preparations used for it, and it might be that a quite considerable grade of allergy remains unnoticed in our test. So we do not think that there is sufficient ground for denying the connection between the hypersensitiveness and resistance, which is supported by good evidence, yet a certain amount of doubt is necessary.

(Dr. H. S. Willis, Baltimore, closing.) It is quite true that the dosage is of great importance. In these experiments all the animals received a constant dose at the time of inoculation, and it was the examination of these animals which had received constant doses, at intervals afterward that the work was based upon. As to whether immunity and hypersensitiveness have their inception at the same time after the preliminary inoculation, I cannot speak with absolute surety. I know that, depending upon the size of the dose, the hypersensitiveness may develop in 6, 7, 8, 9 or 10 days; or with very small doses not until two or three weeks. I know that surely within a few days of the development of the skin sensitiveness the animal is immune. It seems highly probable that they have their inception at the same time. They certainly develop at about the same time and coexist as complements of each other.

NEW METHODS FOR STUDY OF THE SERUM SENSITIZATION OF THE ACID-FAST BACTERIA. Stuart Mudd and (by invitation) Emily B. H. Mudd, Philadelphia.

Abstract. The interfacial tension method (J. Exper. Med., 1924, xl, 633, 647; 1926, xliii, 127) has been used to study the sensitization of acid-fast bacteria. The bacteria are suspended in salt solution and observed with the dark-field microscope at the oil-water interface of a two-phase film. Before sensitization the bacteria are extremely miscible with the oil (tricaprylin, Kahlbaum). Clumps are dispersed explosively by the interfacial tensions and the bacteria are shot violently into the oil. After sensitization with high concentrations of normal

serum or high or lower concentrations of homologous immune serum the bacteria are no longer oil-miscible; they are stable in the oil-water interface; clumps are much more coherent and are markedly resistant to wetting by the oil. This alteration of the bacterial surface by immune serums is specific.

Agglutination tests as ordinarily conducted are notoriously unreliable with the acid-fast bacteria, but may be made more dependable by a simple modification. After the macroscopic agglutination readings have been made in the usual way the tubes are centrifuged. The supernatant fluid is poured off and a few drops of salt solution are added to the sediment in each tube. The tubes are arranged in a rack and shaken uniformly until the control shows an even suspension. The organisms which have been treated with the higher concentrations of serum resuspend in flocculi whether or not they showed agglutination by the ordinary procedure. The size and coherence of the flocculi increases up to the highest serum concentrations even where there was a prezone by the usual method. The interface reaction similarly has shown sensitization of the washed bacilli to be maximal after treatment with serums of maximal concentrations, and the interface reaction is positive with inagglutinable strains. The agglutination prezone and the inagglutinability of certain strains are thus due to inhibition of clumping and not to a failure to bind agglutinins. Agglutinins are bound, but something prevents the bacteria from clumping until they are forcibly brought together in the bottom of the centrifuge tube.

The interface and the resuspension reactions have both been found to be more reliable detectors of the binding of antibodies by the acid-fast bacteria than the ordinary agglutination procedure.

The immune serums used in this study were kindly furnished us by Dr. J. Fürth and Dr. J. D. Aronson.

(Discussion by Dr. H. G. Wells, Chicago.) I would like to ask Dr. Mudd if he considers the absorption of the desensitized bacteria as evidence of the protein character of antibodies. It seemed to be readily interpreted as evidence in favor of that point of view.

(Discussion by Dr. F. M. Huntoon, Glenolden.) I would like to ask a question along the same line. Does Dr. Mudd consider the entire surface of the bacteria to be coated with this material derived from immune serum?

(Discussion by Dr. L. Dienes.) Did you examine the influence of a tubercle bacillus immune serum which had no affinity for the lipoid substances of the bacteria? Such serum can be obtained by the immunization of rabbits with a watery extract of the bacterium. Dr. Freund has found the isoelectric point of tubercle bacillus to be near to the isoelectric point of the protein substances of the bacteria, and to be quite different from the isoelectric point of the lipoids of the bacteria. So, in the coating of the bacterium also, the protein substances seem to play a certain rôle.

(Discussion by Dr. R. R. Mellon, Rochester, N. Y.). I would like to ask Dr. Mudd whether the fact that the antibody being aqueous soluble, and combining presumably with a similar material in the wall of the organism, would be any indication that the surface of the organism itself is really a mosaic of acid-fast material and aqueous material, and not a solid acid-fast surface?

(Dr. Stuart Mudd, Philadelphia, closing.) I think Dr. Wells has very happily expressed the matter. It is an item of evidence which taken with other evidence tends to make one feel that the antibodies probably are protein. It is not conclusive by itself. We must study this interface reaction with a lot of purified chemical substance. We have studied carbohydrate in the form of

shreds of filter paper and of cotton and starch grains. These are the least oil-miscible substances we have found. They stay away over on the water side of the interface. All non-acid-fast bacteria studied are stable in the interface. Oxyhemoglobin crystals and the protein film on oil droplets in milk are stable in the interface; as soon as the film is torn off by the interfacial stresses the milk oil droplet explodes into and mixes with the oil phase. So far as the evidence goes then, this stability in the interface shown by strongly sensitized bacteria is characteristic of protein and not characteristic of the small number of other chemical categories we have studied. But I feel that the number we have examined is too small to allow of any final generalization.

The interface reaction shows that specific agglutination may occur when only a small fraction of the bacterial surface has been coated with antibody. Whether or not the whole surface is coated by the strongest serums, I cannot say.

The work of Dr. Freund I consider pretty conclusive evidence that there is a certain amount of protein in the tubercle bacillus surface. We found a sharp isoelectric point on the acid side of which the bacteria became positively charged. There is no reason to expect a sharp isoelectric point either with the known lipoids or carbohydrates. Lysolecithin and lysocephalin have broad isoelectric ranges, but no sharp isoelectric point. There must be some protein in the tubercle bacillus surface, but I do not in the least agree with Dr. Freund that the surface is all protein.

As regards Dr. Mellon's question, I do not feel that I have any evidence as to what the chemical receptor on the tubercle bacillus surface is. The result of the combination with antibody is the covering up of the surface lipin, but whether the receptor is a part of the lipin radicle or protein or carbohydrate I see no way at present of deciding.

THE CHEMISTRY OF THE COAGULATION OF THE BLOOD. Frank Maltaner and (by invitation) Elizabeth Maltaner, Albany.

Abstract. Certain observations have been made which suggest that the coagulation of the blood is a true example of chemical catalysis and does not involve enzymes. This catalytic reaction seems to have a broad significance, lending support to the speculations of Nolf that the coagulation process is of fundamental importance in the nutrition of the tissues and in the immune and anaphylactic phenomena.

The serozym solutions of Bordet and Delange, supposedly free from fibrinogen and owing their activity to proferment, were shown, when sufficiently concentrated, to contain fibrinogen. The activity of these solutions was apparently due to the fibrin formed as the result of the reaction of this fibrinogen with calcium and cytozym.

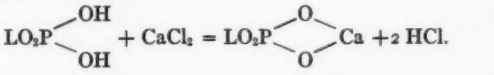
The phosphate plasma of these authors was found to owe its stability to the decomposition of some of the gelatinous tricalcium phosphate used in its preparation, instead of to the adsorption of proferment as they believed. This decomposition consisted in the reaction of the tricalcium phosphate with the sodium chloride of the plasma to produce free alkali and alkali phosphate. The addition of these substances to oxalated plasma conferred upon it all the properties of phosphate plasma.

It was observed that calcium did not function as an activator of a proferment but in reality reacted with the lipoidic constituent essential to blood coagulation to form a relatively insoluble calcium-lipoid compound and liberate acid. It

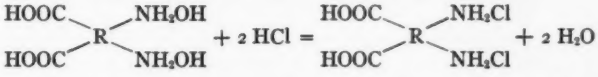
was further shown that not only fibrinogen but all the proteins of plasma when slightly acidified would combine with the active lipoid constituent to form a lipoid-protein complex which would precipitate from solution. When equivalent amounts of lipoid and protein were present the supernatant fluid after precipitation was found to be entirely free from both protein and lipoid.

While calcium in reacting with the lipoidic constituent, in the coagulation of the blood, would not produce sufficient acid to bring about the massive mutual precipitation of the lipoid and protein present, it would initiate a catalytic reaction which under the influence of contact could rapidly produce such a lipoid-protein complex possessing the character of fibrin.

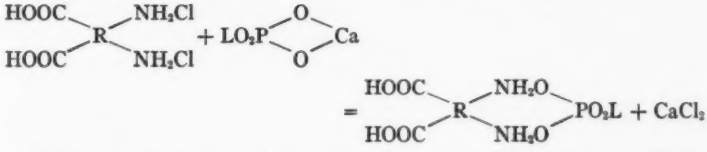
In the coagulation of the blood, the ionized calcium salt initiates the reaction pictured in equation No. 1.



The acid that is liberated reacts with the fibrinogen, with which the blood or plasma is saturated, before it can diffuse from the surface of the precipitated lipoid salt to form fibrinogen chloride as pictured in equation No. 2.



This acid fibrinogen being formed in direct contact with the calcium-lipoid compound reacts with the latter to produce a lipoid-protein complex, which is fibrin, and sets free calcium chloride as illustrated in equation No. 3.



The liberated calcium chloride then reacts with more lipoid and the reaction proceeds to an equlibrium.

These reactions represent a true example of a chemical catalysis in which calcium chloride functions as the catalyzer, under the activating influence of contact. It is suggested that this catalytic reaction explains the mechanism of clot formation.

The lipoid compound obtained by precipitation with calcium chloride in ammoniacal solution possessed a percentage composition similar to that of cephalin containing one calcium atom. It was devoid of coagulating activity but treatment with dilute hydrochloric acid removed the combined calcium and left a compound soluble in alcohol and acetone and possessing the active coagulating function. Reprecipitation of the calcium salt from this material followed by treatment with dilute hydrochloric acid and resolution in alcohol did not affect the coagulating function of the material. The second calcium-lipoid compound, however, was found to be free from nitrogen and possessed a percentage composition equivalent to that of cephalin in which the amino-ethyl-alcohol radical and a hydrogen of the phosphate group were replaced by a calcium atom.

(Discussion by Dr. V. C. Jacobson, Albany.) I should like to ask Mr. Maltaner how, on the basis of his theory of coagulation, he would explain the lengthening of coagulation time following intravenous injections of peptones and foreign proteins.

(Discussion by Dr. H. G. Wells, Chicago.) I should like to ask whether the derivative of the cephalin in Formula 3 when added to fibrinogen in the absence of calcium produces fibrin, as I suppose must be the case according to the theory. I should also like to ask how, on the basis of this theory, the author would explain the common observation of the formation of fibrin when simply a foreign body not active chemically, for instance gold or platinum wire, comes in contact with the circulating blood.

(Discussion by Dr. G. H. A. Clowes, Indianapolis.) I am strongly in favor of this point of view. Ten or twelve years ago I did a lot of work in this field and I regret that I did not put it through as conclusively as this has been done. I am quite satisfied about that calcium-lipoid combination and I would like to ask whether that preparation is found to be heat resistant. I had a substance of this type that would stand boiling half an hour with little loss of activity. The whole picture that we have of lipoid-protein combination in the cell would fit in with this idea and the mere fact of using a needle or anything of that sort and introducing it into a vessel, leading to clotting, would be in harmony with that idea.

(Mr. Frank Maltaner, Albany.) In answer to the question in regard to the introduction of a needle, it is well known that the lipoid substance is contained in mammalian blood in the platelets and contact or the contact with a strange surface attracts the platelets to the strange surface and liberates there the lipoid substance. The calcium and fibrinogen are always present and the reaction is instigated at that surface.

This compound in Formula 3 when added to fibrinogen or when added to plasma — pure fibrinogen probably never has been had — will produce a precipitate. A clot is only formed when the catalytic process takes place.

The matter of heat resistance — the lipoid substance has been shown by all of the modern investigators of the coagulation question to be heat resistant.

(Discussion by Dr. G. H. A. Clowes, Indianapolis.) I do not mean the lipoid substance. I mean the thrombin substance that does the whole thing — the calcium-lipoid complex ready to act.

(Mr. Frank Maltaner, Albany, closing.) That complex — that is, the calcium-lipoid complex — is inactive, has no coagulative function. The thrombin, however, is not heat resistant.

(Dr. G. H. A. Clowes.) Yes, it is.

BACTERIAL SYNERGISM. W. L. Holman, Toronto.

Abstract. The term synergism as applied to bacteriology defines the phenomena of the coöperative action of two or more bacteria which results in the formation of products not formed by the individual bacteria growing alone. The analogous phenomena of the suppression of metabolic products should also be grouped under this term since the products thus formed are also different.

Since 1912 I have been demonstrating to the students in bacteriology the gas producers when these are grown together in a medium containing carbohydrates or similar bodies unfermented by the gas producer. For example *B. paratyphosus* with a lactose fermenting streptococcus gives acid and gas in lactose

broth. Many other combinations were tried and the importance of the results was emphasized in tests for *B. coli;* in picking colonies of *B. paratyphosus* from Endo plates; and in the probable explanation of many altered fermentation tests with the colon-typhoid bacteria where one of the combinations may have died out between the tests. Theobald Smith and D. E. Smith in 1920 reported on the inhibitory action of paratyphoid as distinguished from the hog cholera bacilli on the fermentation of lactose by *B. coli*. Sears and Putnam in 1923 studied the same type of combinations as I had been using and obtained the same results. Castellani, 1925, also reported similar findings.

The present report covers attempts to learn more about the intimate metabolism of the bacteria and considers some of the factors concerned. Many combinations grown in mediums containing substances not attacked by the gas-former result in gas production if the second bacterium is capable of splitting to acid the substance used. The gas-former must also be capable of forming gas from glucose.

B. communis (non-saccharose fermenter) and *Streptococcus fecalis* were used in the more detailed study in plain extract broth with 1 per cent saccharose.

The two bacteria may be growing together and fail to give gas if the relative numbers of each bacterium are not suitable or if the hydrogen ion concentration does not favor gas production. All grades of gas production can also be obtained by varying the numbers of each organism in the seedings and the time of the addition of the second bacterium. This was shown to be partly due to differences of the two organisms in their rates of growth, the *B. coli* reaching its maximum in about half the time required for the streptococcus, and further to the differences in the pH value at different periods. The two bacteria must be living in close association. Filtrates of either or both organisms did not give gas when seeded with the other organism. When grown in the above medium in U tubes closed at one end and partly filled with sand there was no gas formed until both bacteria had penetrated the sand.

There is less carbon dioxide in the gas produced from saccharose by the combination than that formed by *B. coli* alone from glucose, the synergistic ratio being $\frac{H}{CO_2} = \frac{4}{1}$, that of the *B. coli* alone $\frac{H}{CO_2} = \frac{1.5}{1}$. This would suggest that part of the CO_2 was retained in the medium as carbonates from the action of the *B. coli* on the organic acids. Ammonium lactate in broth is acted upon by *B. coli* with alkali production and added to dextrose broth lessens the amount of acid and gas produced by this organism.

There are thus two opposite processes going on and the question of whether gas will be produced or not depends on which process is more active at the time in the metabolic life of the bacterium. It is to be emphasized that the above conditions may not infrequently occur and many errors in interpreting the results of fermentation tests can be thus explained. It is of importance in water work, isolations from stools and in general studies where fermentation tests are employed. The careful study of these synergistic reactions will lead to a better understanding of the metabolism of bacteria.

(No discussion.)

A Pathogenic Bacillus Resembling the Diphtheria Bacillus. Ruth Gilbert and (by invitation) F. Constance Stewart, Albany.

Abstract. A pathogenic organism resembling the diphtheria bacillus and similar to that described by Parker has been isolated from twenty-five throat cultures

received at this laboratory. In young cultures the organisms resemble diphtheria bacilli, polar bodies being readily demonstrated, while in older cultures coccoid forms predominate. The cultural characteristics are similar to those of diphtheria bacilli except that gelatin is liquefied in six to seven days and nitrates are not reduced.

Filtrates of broth cultures were found to be toxic and a horse was immunized with them. After thirteen months of immunization, 1/150,000 cc. of its serum neutralized 0.01 cc. of the toxic filtrate when injected intracutaneously in rabbits. Before immunization the serum of the horse contained less than 1/500 unit of diphtheria antitoxin per cc. but after immunization as much as thirty units were present at one time. One-hundredth cc. of the toxic filtrate was found generally to be neutralized by 2.5 units of diphtheria antitoxin. These results would indicate that the organism has some immunologic relation to the diphtheria bacillus.

(Discussion by Dr. M. A. Goldzieher, Budapest.) May I ask whether there was any difference in the morphology of the bacillus with Gram stain or with any other stain, for instance methylene blue? I have studied the bacillus of inguinal granuloma, the morphology of which is very similar to this one. This bacillus took on a coccoid form with the Gram stain but if stained with methylene blue without using alcohol or any other fat solvent substance then the bacillus form was still visible. I therefore conclude that there was a fatty degeneration in the body of the microörganism and this fatty substance which I think is a fatty acid was dissolved by absolute alcohol or other fat solvent substances which I used. May I ask if such observations were made in this case?

(Miss F. C. Stewart, Albany, closing.) The organisms we studied were gram-positive and showed the same changes in morphology at the different incubation periods with the Gram stain as was noted with Loeffler's alkaline methylene blue stain. We did not use an aqueous methylene blue.

THE PASSAGE OF BACTERIA FROM THE PERITONEAL CAVITY INTO CAPILLARIES AND LYMPHATICS IN COLON BACILLUS PERITONITIS. Bernhard Steinberg (by invitation) and Harry Goldblatt, Cleveland.

Abstract. Simultaneous cultures of blood (femoral artery) and lymph (thoracic duct) were taken after the intraperitoneal injections of (*a*) *B. coli* suspended in physiological saline and (*b*) *B. coli* suspended in gum tragacanth.

In the experiments with saline suspensions, the bacteria appeared in lymph and blood, a little earlier in the former. Estimation of the number of bacteria showed a great preponderance in lymph.

In the experiments with gum tragacanth suspensions, bacteria appeared in lymph but not in blood. The number of bacteria in this lymph was very much smaller than in the lymph after the injections of saline suspensions.

Dogs injected intraperitoneally with *B. coli* and gum tragacanth developed a hemorrhagic sero-fibrinous peritonitis and died. Dogs injected intraperitoneally with *B. coli* suspensions in saline showed a slight hyperemia of the peritoneum, developed a bacteremia but survived.

It is suggested that the fatal results after injection of gum tragacanth suspensions may be due to the elaboration of the *B. coli* toxin in the peritoneal cavity. This phase of the work is being continued.

(Discussion by Dr. Stuart Mudd, Philadelphia.) Did you attempt to see if the gum tragacanth opsonized the bacteria?

(Dr. B. Steinberg, Cleveland, closing.) No.

UNDULANT FEVER IN MAN ASSOCIATED WITH BACTERIA INDISTINGUISHABLE FROM BRUCELLA ABORTUS. V. A. Moore and (by invitation) C. M. Carpenter, Ithaca.

Abstract. The purpose of this paper is to describe a few cases of undulant fever in man caused apparently by infection with the organism of the Bang abortion disease and to point out the similarity that exists between it and Malta fever. The relation of *Brucella melitensis* to the organism found in the cases of undulant fever is like that between *Br. melitensis*, found occasionally in goat's milk, and *Br. abortus*, sometimes found in milk of cows that have aborted. A brief review of cases of human infection with this organism have already been published by Keefer, DeKorte, Orpen and others and the results of the tests for antibodies of *Br. abortus* in human blood. A brief review of the cases is supplemented by six cases of undulant fever in which *Br. abortus* was isolated from the blood. Abortion was produced in pregnant heifers with cultures isolated from four of the cases. The inoculation produced a more severe reaction in cattle than usually follows the injection with *Br. abortus* isolated from infected cows. There are no cases of abortion reported in the human species in which this organism has been established as the cause. *Br. abortus* was isolated from cows' milk in 1911 and 1912 by Schroeder and Cotton and Smith and Fabyan, and since that time it has been found frequently in the milk of herds in which abortion exists. The source of infection in the human cases reported is not determined. It is known that two of them drank freely of raw milk from herds where abortion in the cattle had occurred and in the milk of which the organism was found. A study is being made of the bacteria in market milk in reference to the prevalence of this organism and the virulence of the different strains. As a precaution pasteurization of milk is recommended.

(This paper discussed with the next paper.)

A COMPARISON OF STRAINS OF BRUCELLA ABORTUS ISOLATED FROM MAN WITH THOSE FROM CATTLE. C. M. Carpenter (by invitation), Ithaca.

Abstract. The author has isolated five strains of *Brucella abortus* from the blood and urine of men suffering from undulant fever. The cultures were isolated by sealing the tubes with wax and subjecting the cultures to an atmosphere where 15 per cent of the air was replaced by 10 per cent CO_2. Cultures were positively identified as *Brucella abortus* by the agglutinin absorption test. The cultures isolated from man were compared with those from cattle with regard to the following characters: atmospheric requirements, serologic relationships, pathogenicity for guinea-pigs, their effect upon pregnant cattle when injected intravenously, and their ability to establish themselves in the udders of cattle.

The results of these studies disclosed the following facts. The first generation of the human strains was always very difficult to grow, but the second and following generations grew well upon unsealed tubes of nutrient agar. Certain bovine strains which the author has studied have shown this same characteristic while others have, for longer or shorter periods of time, required an increased amount of CO_2 and some enriching substance in, or added to, the medium, such as sterile blood or serum. The cultures isolated from men have been extremely virulent for guinea-pigs, but not more virulent than certain strains of *Brucella abortus* isolated from bovine sources. Four of the five human strains have produced abortion in pregnant heifers when injected intravenously. The fifth strain was injected only very recently, but, judging from the symptoms the animals is showing at present, she will abort. The organism has been recovered

from the fetuses, placentas and milk of the four cows which have aborted. One strain of *Brucella abortus* of human origin established itself in the udder of a heifer and remained there for six and one-half months when the animal was destroyed. The cultures isolated from man were more toxic for the pregnant heifers than were bovine strains and produced abortion in a shorter period of time.

(Discussion by Dr. James Ewing, New York.) Will Dr. Moore kindly state if there was any involvement of the lymphatic system in this infection in man or animal? Has the condition any resemblance to the so-called glandular fever? Was the structure of the glandular lesion in cattle that of suppurative inflammation, or of a lower type of inflammatory reaction?

(Dr. V. A. Moore, Ithaca.) We find in the calf some enlargement in the presence of the organism in the lymph glands. It is quite possible that glandular fever may be due to this organism but it has not been considered in our studies and we have not carried the work far enough to draw conclusions on this point. In regard to the udder changes, we have not finished our studies on that subject. Most of the cases of abortion in cattle do not come to postmortem and we do not have a chance to study the udders histologically. We are undertaking now a study not only of the changes in the udder but also of the cellular content of the milk which may be somewhat different from that of ordinary pyogenic infections.

(Discussion by Mr. J. G. Olson, Indianapolis.) There are a number of well known foci in this country, particularly in Texas, Arizona, New Mexico and Utah from which Malta fever might be disseminated. In the spring of 1921 we were able to obtain histories of something more than fifty cases of definite Malta fever which had occurred in human beings in Utah, over a period of about fifteen years, with six deaths. This mortality was unusually high. There are several foci in Utah where goat's milk is produced and cheese is made from the unpasteurized milk. The cheese is sent to New Orleans and other places. Even as tularemia is moving eastward so from such foci of infection we may find Malta fever spreading over the country and this work serves to call to mind the fact that undulant fever should be followed up bacteriologically very carefully and undoubtedly in the near future many more cases will come to our attention.

(Discussion by Major James Coupal, Washington.) I would like to know if any of the authors' cases occurred in the region of Plattsburg, N. Y., or Fort Ethan Allen, Vermont.

(Dr. V. A. Moore, Ithaca, closing.) There were no cases from Plattsburg or Fort Ethan Allen. Goats do suffer from abortion the same as cattle. I have a verbal report of one instance of this trouble in the Southwest (Arizona) where 50 per cent of the pregnant goats in a herd aborted. The cause was not determined but it is presumable that it was due to one of the Brucella as the history indicated that it ran a course similar to the Bang abortion disease in cattle.

EPIDEMIC HICCUP: ANIMAL EXPERIMENTS AND MICROSCOPIC LESIONS OF BRAIN AND CORD IN ONE CASE. E. C. Rosenow and (by invitation) Harry Parker, Rochester, Minn.

Abstract. The patient, a man 68 years of age, while in the hospital prior to a contemplated prostatectomy for hypertrophy of the prostate at a time when a mild epidemic of hiccup was occurring, developed fever, inability to talk above

a whisper, difficulty in swallowing, intermittent hiccup, vomiting, restlessness, marked generalized weakness and difficulty in breathing with choking and syncopal attacks, in one of which he died two days after onset of symptoms.

Neurologic examination by Dr. Harry Parker revealed loss of the left corneal reflex, a crossed anesthesia of the left side of the face and the right side of the body and the findings of a left vagoglosso-pharyngeal hypoglossal paralysis indicating lesions in the left side of the upper medulla, involving the nuclei of the ninth, tenth, eleventh and twelfth cranial nerves and the spinal root of the fifth nerve.

Necropsy revealed chronic sinusitis, nasal polypi, hypertrophy of the prostate, marked arteriosclerosis, especially of the cerebral vessels, and retrograde thrombosis of the left posterior inferior cerebellar and vertebral arteries. No gross lesions of brain and cord were found.

On account of the intermittent attacks of hiccup and the occurrence of a mild epidemic of singultus at the time, it was thought that possibly the symptoms might be attributable to the streptococcus which has been identified with epidemic hiccup and encephalitis. Accordingly, cultures and animal inoculations were made with swabbings from the nasopharynx, from the catheterized urine during life, and from the blood and urine after death. The streptococcus identical to the one isolated in epidemic hiccup and which produced spasms of the diaphragm or other muscles, associated with bulbar symptoms, in inoculated animals, was obtained from nasopharynx, urine and blood. It was isolated in pure culture from the brain and medulla and demonstrated in the lesions in the animals that developed characteristic symptoms.

Microscopic sections of the brain and cord of the patient revealed two poorly staining areas of infarction on the left side of the medulla, the larger in the posterior and the smaller in the anterior aspect in the region of the olive. These were largest at about the level of the vagus nucleus and rapidly diminished in size above and below this level. In these areas there was found thrombosis of vessels, light staining and degeneration of nerve cells, leucocytic and round cell infiltration and slight perivascular infiltration by leucocytes and round cells. No noteworthy lesions were found in sections taken from various parts of the brain, pons and spinal cord. Gram-positive diplococci, singly, in groups and sometimes in chains of two, were found within and adjacent to the lesions in the medulla and within one of the thrombosed blood vessels. No bacteria were found in sections remote from lesions.

(Discussion by Dr. James Ewing, New York.) How long after the operation did the patient die? How long after death was the autopsy performed?

(Dr. E. C. Rosenow, Rochester, Minn., closing.) The patient was not operated upon. He was merely in the hospital being prepared for prostatectomy. The autopsy was held two hours after death.

MICROSCOPIC LESIONS OF THE CENTRAL NERVOUS SYSTEM IN RABBITS INJECTED WITH STREPTOCOCCI FROM ENCEPHALITIS, SPASMODIC TORTICOLLIS AND POLIOMYELITIS. E. C. Rosenow and (by invitation) M. Balado, Rochester, Minn.

Abstract. The relationship between the symptoms and the lesions found in rabbits after injection of virulent material of epidemic encephalitis, is an object of discussion because spontaneous encephalitis of rabbits with its typical anatomic pathologic picture rarely gives rise to symptoms.

In an extended study of the effects of inoculation of the streptococcus isolated in encephalitis and allied conditions, marked discrepancies between symptoms and lesions were sometimes noted, especially as regards the late manifestations or so-called sequellae. With the object of establishing this point, we have examined serial sections of the brains of three rabbits that showed characteristic symptoms. One of these (R 1080) was injected 160 days previously with the streptococcus from the nasopharynx in a case of spasmodic torticollis; one (R 1174) eighty-four days before with a suspension of the streptococcus from poliomyelitis and the third, thirty-one days before with a green-producing streptococcus from milk and which produced encephalitis in rabbits.

Rabbit 1080. The head always tilted and rotated to the left to approximately an angle of 90 degrees. The right eye looked up, the left down. The left pupil was larger than the right. When the animal was held vertically in palm of hand, the abnormal position of the head increased; when held over the back with head downward, right side of body rotated to the anterior position and the head became completely rotated. The abnormal rotation was corrected when the animal was lifted by the ears.

Rabbit 1174. When quiet in cage, animal appeared normal. When prodded, it always went backward. When held upright in the palm of the hand, the head always was tilted to the left. The left fore extremity was extended and the right flexed. Hanging with the head down, the head was drawn to the left.

Rabbit 1213. Symptoms were similar but more marked than those in Rabbit 1080, but torsion of the head and body was to the right instead of to the left. Under excitement, horizontal nystagmus occurred. The pupils reacted slowly to light. The position of the body and head was not corrected when the animal was lifted by its ears.

The animals were all injected intracerebrally, well forward in the right frontal lobe and were killed with chloroform. No microscopic changes were found at necropsy. The sections were stained by Nisal's method, as modified by Leukossek and Jakob of Buenos Aires and examined for lesions throughout the brain substance, paying especial attention to their distribution, especially with regard to the nucleus of the vestibular nerve.

Widely disseminated foci of lymphocytic infiltration were found in Rabbits 1080 and 1174. These were most numerous in the cortex, in lesser numbers in the thalamus, and cerebral peduncles and only rarely occurred in the pons. They were bilateral and showed no relation between their location and the symptoms of the rabbits. The nerve cells, especially in the brain stem were normal in size and staining reaction. In Rabbit 1213, the one with the most marked postural symptoms, no foci of lymphocytic infiltration were detected and the nerve cells appeared normal throughout.

In four other rabbits with similar symptoms, search for lesions was made in frozen sections stained by the method of Spielmeyer, for myelin. No lesions were found of the vestibular nerve.

On the basis of these findings, we would conclude that with the methods employed, we cannot detect changes in nerve cells that explain the particular symptomatology in the rabbits examined and that the foci of lymphocytic infiltration (the typical histologic picture of spontaneous encephalitis in rabbits) have nothing to do with the symptoms induced by the injection of the streptococcus of epidemic encephalitis and allied conditions.

(No discussion.)

THE FILTERABILITY OF THE MOUSE SARCOMA AND CARCINOMA. M. J. Sittenfield, New York City.

Abstract. Gye's report, setting forth his conception of the etiology of cancer, aroused so much interest that it became most essential to confirm his experimental evidence. For this purpose mouse sarcoma No. 37 was used. The injection of the supernatant fluid from anaerobically incubated cultures of mouse sarcoma No. 37 yielded in our hands 32 per cent of tumor takes. The tumors appear on the average seventeen days after injection. Microscopically, they closely resemble the parent tumor. As a control to this experiment the possibility of cells remaining alive in the fluid was tested by inoculating the incubated tumor tissue itself. Out of sixty-three inoculations of washed tissue, there were but two takes, out of fifty-eight inoculations of unwashed tissue there were three takes. If our results are due to the presence of live cells it is difficult to reconcile the 32 per cent of takes from the supernatant fluid with the very small percentage of takes obtained from the inoculations of the incubated tumor tissue itself.

The acid test of ruling out the presence of cells in the incubated fluid was met by the filtration of the fluid through a Berkefeld filter "N," which had been tested against bacteria before and after filtration.

For the mouse carcinoma No. 63 we claim only two takes from the supernatant fluid, none from the filtrate. We are thus able to confirm Gye's experiments concerning the filterability of mouse sarcoma No. 37, which yielded in our series of experiments eight tumor takes out of 183 mice, or about 4 per cent of takes in the mice injected with the cell free filtrate of mouse sarcoma No. 37.

(Discussion by Dr. I. P. Lyon, Buffalo.) In this connection I recall some experiments that I saw reported in the Journal of Parasitology about a year or so ago in which some one had succeeded in filtering trypanosomes and securing successful takes from the filtrates by the material going through Berkefeld filters of some sort, I do not know the size. I was very much astonished to see that enough of the trypanosomes could go through in order to grow. It would seem it is possible that a cell, such as a trypanosome, possibly as large as a tumor cell, does send something through a Berkefeld filter which insures growth when placed in a suitable environment. Such a conception of a cell is beyond our ordinary ideas but that work on trypanosomes and this work referred to in regard to the filterability of tumors suggests that possibility. It seems to me it is quite as probable that something goes through from the tumor cell, which enables the cell to reproduce, as that it is a filterable virus (about which we know nothing) which goes through.

(Discussion by Dr. W. L. Holman, Toronto.) As shown in the paper to be read by title later, we tried a number of filter candles by testing the amount of fluid that went through in a given time. If we oiled them with vaseline or a mixture of paraffin oil and paraffin, they became very much more permeable to bacteria and less permeable to fluid. In other words, we did away to a great degree with the adsorption on the surface of the pores of the filter. This may be interesting in regard to the possibility of filtering substances adsorbed on the surfaces of the clean or unoiled filter.

(Discussion by Dr. Stuart Mudd, Philadelphia.) It is not a simple matter. It is very far from being simple. H. Rechhold published a formula by which the size of the Berkefeld or other filters could be estimated. Applying the formula in its original form we reach the conclusion that the diameter is about 0.4 micron.

As pointed out by Bigelow and Bartell (J. Am. Chem. Soc., 1909, xxxi, 1194) a factor in the denominator of Berkefeld's formula was ten times too big. That brings the actual intergranular diameters of clean Berkefeld filters up to about 4 microns. That is a pretty big space. The difference in the three types of filters (V, N and W) is not in the intergranular spaces but in the amount of coarse pores present. This very interesting report of Dr. Holman shows, as many of us have concluded from other evidence, that the pores of a clean filter were plenty big enough to let through bacteria. Indeed, Wolbach, using spirochetes whose filterability had been enhanced by long continued selection, was able to demonstrate them in the filtrate immediately after passage through a Berkefeld filter. Berkefeld filters appear to owe their tightness for bacteria (1) to the tortuosity of the channels through them; (2) to the fact that adsorption of protein or other matter from the filtering fluid quickly reduces the diameters of the intergranular spaces; adsorption is less if the filtering fluid is weakly alkaline than if it is acid — *i.e.*, on the alkaline side of their isoelectric points, the ampholytes present are negatively charged and hence do not so readily adhere to the electro-negative filter pore walls; (3) probably also adsorption of the bacteria themselves on the filter pore walls play a part; (4) if the filtration is continued for a considerable time a "cake" forms on the outside of the candle and is in itself a filter. No doubt other factors also influence the filtration process. Certainly we cannot conclude, as is so often done, that because things go through a Berkefeld filter they are below the dimensions of microscopic visibility.

(Discussion by Dr. R. R. Mellon, Rochester, N. Y.). I want to call attention to the fact in connection with trypanosomes passing through the filter, it is not a bad idea to remember that an increasingly large number of organisms are being found to have filterable stages in their life history. I recall that eight years ago we were repeatedly able to pass a filterable organism from the blood serum of the patient's blood through a filter. This organism grew then in the filtrate as a staphylococcus which would not go through the same candle, and yet we could repeatedly pass diluted specimens of this blood serum through this filter candle. It is claimed too that tubercle bacilli have a filterable stage.

(Dr. M. J. Sittenfield, closing.) It is not my intention at this time to discuss the presence of a microörganism in cancer. In our present report we make no claim that we are dealing with a microörganism in the filtrate. At any rate, it seems to me most improbable that cancer cells could have passed through a filter. It may be interesting to relate in this connection that for about two months and a half our attempts to get a tumor from filtrates were negative, until we refined our technic, eliminated chloroform or ether as an anesthetic, etc., and perfected the anaerobiosis. It was found that by protracted exposure of the culture fluid to the air during filtration we were not able to get a tumor, and only by revising our technic and excluding the air as much as possible during the time of filtration were we then able to get 4 per cent of tumors from the filtrate in the injected animals.

THE CAUSE OF DEATH IN CARCINOMA CASES. Margaret Warwick, St. Paul.

Abstract. This is a statistical study of 560 cases of carcinoma found in the 7900 necropsy reports on file in the Department of Pathology at the University of Minnesota. These cases were analyzed from the point of view of the major complications of the carcinoma, particularly in regard to the immediate cause of death. Of these, carcinoma cases of pancreas, gall bladder, esophagus,

larynx, pharynx, duodenum, and bladder, all showed death to be caused by major complications, and not the tumor growth alone. But the carcinoma alone caused death in 2 or 6 per cent of the cases involving prostate, 1 or 58 per cent of malignant melanomas, 8 or 53 per cent of ovary, 15 or 42 per cent of the lung, 3 or 7 per cent of rectum and sigmoid, 4 or 16 per cent of uterus, 15 or 39 per cent, and 25 or 14 per cent of the stomach. Bronchopneumonia was the most frequent cause of death in carcinoma of the esophagus, ovary, lung, pharynx, breast, and in malignant melanoma. Peritonitis was the most frequent cause in carcinoma of colon, rectum and sigmoid, bladder and stomach. Hydronephrosis was the most frequent cause in carcinoma of prostate and uterus. Obstructive jaundice was the most frequent in carcinoma of the pancreas and gall bladder.

(Discussion by Dr. Alfred Plaut, New York.) I would like to ask whether all the patients who died, died from cancer alone. Were they emaciated or were there some who showed a good amount of subcutaneous fat, for instance? I have seen cases where no direct cause of death could be found.

(Discussion by Dr. M. A. Goldzieher, Budapest.) I would like to call attention to one point which seems to have been disregarded. It is well known that the heart muscle in many cancer areas is subject to fatty degeneration with consequent dilatation of the heart, which may account directly for the death. I think that in these cases of "carcinoma only" quite a few were due to such heart failures. Anemia is a much more frequent cause of death in cancer than these statistics would show.

(Dr. M. Warwick, St. Paul, closing.) There seems to be no rhyme or reason about the emaciation. A great many of these patients were markedly emaciated and a great many were not, but those who died of carcinoma alone were not more emaciated than were the others, and I found that a few patients dying from carcinoma with metastases were well nourished.

As far as the condition of the heart muscle goes, I feel confident that a number probably died from heart failure. It is impossible to measure the exact degree of involvement of the heart muscle at the autopsy so I did not include that in the causes of death. However, many of them were listed as having dilatation of the ventricle or myocarditis and these probably died of heart failure. The same is true of anemia. I feel sure that many of these patients died of anemia but there is no way of determining this at the autopsy alone, and since we cannot always get full clinical notes I did not include anemia as a cause of death unless I knew definitely that it had been very pronounced during life.

A few of these carcinoma cases died an accidental death, and one was suicide.

Histological Picture and Prognosis in Carcinoma of Uterine Cervix.

Alfred Plaut, New York.

Abstract. Three different problems are presented: first, predicting the clinical course from the microscopic picture in any tumor; second, the same for one definite group of tumors; and third, regarding a certain kind of tumor in a certain organ. Further problems are grouped around the words "degree of malignancy" especially when established from a "cell type."

Among many cases which were studied 150 offered data of sufficient exactitude. An attempt was made to follow Martzloff's classification ("spinal cell, transitional cell, fat spindle cell"). The examination of the slides preceded

the study of the clinical course. We did not, however, find a definitely better expectancy of cure in the groups which are supposed to be less malignant. Only for the cases with hornification the surviving rate was higher. Spinal without hornification showed even lower survival rate than the "transitional" cases which are considered more malignant by Martzloff. No group of real "fat spindle cell" carcinoma could be established. Among nine cases in which the presence of fat spindle cells was conspicuous, four were living and well after three years. Thus a tumor containing many spindle cells does not mean necessarily an unfavorable course of the disease. The question of adenocarcinoma was omitted in this paper for lack of sufficient data. The distribution of our material according to the groups of Martzloff would be: 108 spinal, 22 transitional, 10 adenocarcinoma, 1 spindle cell carcinoma and 8 undefinable. A more differentiated grouping would be:

30 spinal with hornification
56 spinal without hornification
5 spinal and transitional
10 between spinal and transitional
7 transitional and spinal (some with horn)
22 transitional (including wide variations)
10 adenocarcinoma
1 spindle cell carinoma
8 undefinable

The borderline between spinal and transitional has been drawn differently by Martzloff and by us. This difference does not interfere with the comparison of results. Cases with five year cure and cases with death within one year showed the same distribution among the histologic groups. The cases with three and four years' cure had the same number of spinal that all cases had. The surviving rate did not show a higher figure for the spinal cases, but the same as for transitional. This lack of difference between his groups somehow has been stated by Martzloff himself. He says that tumors belonging to different groups grow through the uterine wall with the same speed; and he saw no cure after true broad ligament involvement. The consideration of all this together shifts the prognosis again to the clinician. In ten slides the histologic picture was very irregular, one belonged to a patient who lived for two and one-half years after onset of symptoms; all the others died within one year. In such striking cases histologic prognosis can be possible. No other single histologic feature was found in direct relation to the outcome and no group of such features either. It is the same with the results of clinical pathology.

Undifferentiated cells are considered to indicate a highly malignant tumor. Since they are on the other hand highly susceptible to radiation another difficulty is introduced into histologic prognosis.

The cellforms in cervical carcinoma are so manifold that grouping seems a hopeless task.

Conclusions: (1) We have no reliable basis for histologic prognosis in cervical carcinoma.

(2) The cellforms of cervical carcinoma do not admit of establishing well-defined groups.

(3) In speaking about malignancy the radiosensibility must be considered separately.

(4) The clinical classification is still the best aid in making a prognosis.

(Discussion by E. T. Bell, Minneapolis.) Our experience has been somewhat the same as that of Dr. Plaut. We cannot form an accurate idea as to the malignancy of the tumor on the histologic structure alone, except in the case of markedly undifferentiated tumors which are more malignant. The extent of the tumor at the time the treatment is instituted is of much more value in prognosis.

(Discussion by Dr. James Ewing, New York.) There is much value in emphasizing, in the minds of pathologists and surgeons, the great variation in the prognosis of cancers, and the considerable dependence of this prognosis upon structure.

Recently, I have been going over a series of mammary cancers from this point of view. All the relatively benign cases were picked out successfully and all the very malignant ones. There was, however, an intermediate group in which many errors were made in predicting the outcome on the structural type. It became evident that these errors would have been greatly reduced, if attention had been paid to the duration and extent of the disease, at the time of the operation. In this large group of cases, it seems to me unwise to attempt to establish a prognosis on structure alone.

I agree with Dr. Plaut that every case of cancer should be regarded as a special study, on its own merits.

(Discussion by Dr. F. B. Mallory, Boston.) I would like to bring up one point not yet mentioned. If you find one or two mitotic cells you know your tumor is growing. If a dozen, you know it is growing rapidly. That is the point on which you want to base your prognosis as to whether the case is going to end quickly or not.

(Dr. Alfred Plaut, New York, closing.) I must apologize to the members that I omitted certain figures. This material has been fixed after removal and one section may have been fixed shortly after and another one much later. The fixative was formalin. I could not find a direct relation between the number of mitotic figures, irregular or regular ones, and the outcome of the disease. I spent a good deal of time in doing this and I had to give it up. I spoke only about the carcinoma of the cervix. I do not know whether it will be possible to establish the kind of diagnosis in other kinds of cancer and in other organs.

THE SOLID TYPE OF OSTEITIS FIBROSA. J. S. McCartney (by invitation), Minneapolis.

Abtsract. Of seventeen cases of osteitis fibrosa on record in the Department of Pathology, University of Minnesota, four are of the solid type. These four cases are presented.

The first involved the external condyle of the femur in a male aged 35. Trouble present two months. Condyle curetted and cauterized. Sections of the material showed compact hyaline connective tissue, apparently growing very slowly. Three months later, on account of suppurative arthritis and osteomyelitis, the leg was amputated. The specimen showed fleshy tumor tissue protruding from the external condyle, grossly looking like sarcoma. Sections of this showed an actively growing connective tissue with frequent mitoses.

The second case, a woman aged 53, had pain in the knee for several years following injury. Leg amputated ten years ago with diagnosis of osteosarcoma. A tumor about 7 cm. in length and 5 cm. in breadth involved the center of the tibia. The tumor was grayish white in color and of firm consistence. Sections

showed loose and compact connective tissue with rather frequent mitotic figures. In addition there was perforation of the cortex and appearance of fibrous tissue beneath the periosteum.

The third case, a female aged 15, had pain for eighteen months following injury. The base of the femur was rarefied. Cavity curetted. Material from the cavity was loose connective tissue. Sections showed in addition active destruction of bone by osteoclasts. Curettage done two and a half years ago. At the present time the condition is progressing and there is also involvement of the ilium.

The fourth case, a woman aged 38, had pain in the foot for twenty years. X-ray showed involvement of the cuboid and possibly of the cuneiform. Material from this area showed loose connective tissue.

Sections of the tissue from Cases 1 and 2, particularly, could readily be diagnosed sarcoma. Such cases as these have been amputated and reported as cures of osteogenic sarcoma.

(Discussion by Dr. James Ewing, New York.) I would like to ask if any of these cases would get well if treated by moderate repeated doses of X-ray.

(Dr. J. S. McCartney, Minneapolis, closing.) The girl of fifteen is the only one of our series who showed involvement of more than one bone (femur and ilium). She has been getting X-ray treatment ever since the operation in July 1923 and the disease is extending. The head of the femur is expanding and the areas in the ilium are gradually getting larger.

ON THE PATHOLOGY OF CATARRHAL JAUNDICE. Paul Klemperer and (by invitation) John A. Killian, New York.

Abstract. Clinical observations and various laboratory tests for disturbed liver function indicate that the functional capacity of the liver is severely altered in cases of so-called catarrhal jaundice. These facts are suggestive of anatomic changes in the liver parenchyma rather than inflammatory changes either in the large or small bile ducts. Owing to the benign nature of the disease anatomic data are extremely scarce. Only necropsy findings in the four cases reported by Eppinger can be accepted.

We have had the opportunity to observe a case of so-called "catarrhal jaundice" where an exploratory laparotomy was performed. The exploration of the bile ducts, gall bladder and pancreas gave no explanation for the intense jaundice. The liver was large but did not show biliary stasis. A small piece was excised for diagnosis. The outstanding microscopic findings were diffuse, severe parenchymatous degeneration with necrosis, primarily localized within the center of the acini. There was no cholangitis. The intracellular bile capillaries, however, showed ruptures due to the liver cell necrosis.

These morphologic findings corroborate the modern clinical conception that the so-called catarrhal jaundice is due to an injury to the liver cells.

(Discussion by Dr. M. J. Stewart, Leeds, Eng.) During the war, we saw in Leeds many cases of trinitrotoluene poisoning. We did eight or ten postmortems and in every one there was an acute or subacute atrophy of the liver. Some five years later I traced all the patients who had been in the Leeds Infirmary suffering from trinitrotoluene poisoning with jaundice, and who made recovery and all were in excellent health. In a case of salvarsan (Galyl) poisoning on which I made a postmortem I came to similar conclusions to those of Dr. Klemperer and Dr. Killian. A girl came to the venereal disease clinic with

syphilis and was treated with a course of five injections. At the end of the course she had a slight attack of jaundice which lasted three or four days. She came back to the hospital two months later to have venereal warts removed, and died twelve hours after operation. At postmortem she had typical subacute atrophy of the liver with scattered red areas of total destruction, while the rest of the organ which had been in the yellow stage was intensely fatty. That (major) portion of liver had survived the toxic onslaught and she was recovering but the undestroyed liver had been getting fatty and was unable to withstand the anesthetic. In the ordinary way this case would have been diagnosed as catarrhal jaundice. It was really one of acute atrophy of the liver with limited destruction of parenchyma in which recovery would almost certainly have occurred but for the using of a general anesthetic.

(Discussion by Dr. E. B. Krumbhaar, Philadelphia.) I would like to ask Dr. Killian the nature and extent of the van den Bergh reaction, if it was done, and also the condition of the Kupffer cells which, from the slides, were not involved.

(Dr. J. A. Killian, New York, closing.) In regard to the question just asked, that will be presented in the next paper.

CONTRIBUTIONS TO LIVER PATHOLOGY IN ACUTE YELLOW ATROPHY AND FATAL PHOSPHOROUS POISONING. John A. Killian (by invitation) and Paul Klemperer, New York.

Abstract. In both cases presented, the diagnosis was based upon postmortem findings, and in the case of phosphorus poisoning, phosphorus was demonstrated in the liver. In the three specimens of blood obtained from the acute yellow atrophy during the twenty-four hours before death in the hospital, there was noted a progressive rise in the nonprotein nitrogen of the blood. The amino and rest nitrogen of both the blood and urine was increased, but the urea nitrogen of the urine formed but 60 per cent of the total nitrogen. In the first specimen of blood, the urea nitrogen was within normal limits, but formed only 30 per cent of the nonprotein nitrogen. In the later specimens the urea nitrogen was increased above normal but formed but a small fraction of the nonprotein nitrogen. The ammonia nitrogen of urine was increased in proportion to the total nitrogen and this was associated with a mild acidosis. At the height of the toxemia there was observed a hypoglycemia. The infusion of 100 gm. of glucose produced a marked hyperglycemia persisting for more than two hours and accompanied by a glycosuria. In all specimens of blood the fibrin was low, but in the specimen obtained before glucose infusion no fibrin could be obtained from blood plasma. The patient had a marked icterus with a high icterus index and strongly positive van den Bergh reactions.

A child of 5 years was admitted about thirty hours after onset of sickness and about two hours before death from phosphorus poisoning. The blood and urine obtained upon admission show a low urea nitrogen in relation to total nonprotein nitrogen, high amino nitrogen, and (in the urine) ammonia nitrogen. There was a marked hypoglycemia and at this time the patient was in convulsions. The icterus index was increased to about eight times the normal, with a very strongly positive indirect, and a positive direct van den Bergh reaction.

(No discussion.)

THE PATHOGENESIS OF CIRRHOSIS OF THE LIVER. M. A. Goldzieher (by invitation), Budapest.

Abstract. A series of cases of liver cirrhosis has been studied. It has been observed that there are constant anatomic findings in some of the endocrine glands. The thyroid and the gonads are, as a rule, decreased in size. There is an excess of stroma in the thyroid and changes in the glandular parenchyma similar to atrophy. In the testicles beside the atrophy of the seminal tubules and hyalinization of their tunica propria, there is a hyperplasia of Leydig's cells present. The latter findings are identical with those in congenital underdevelopment. In the ovaries the changes found are comparable to those in the testicles.

The pineal of the cirrhotics is constantly larger in size and does not show those signs of involution which are regularly found in individuals of mature age.

There were less constant anatomic findings in other endocrine glands such as the hypophysis, adrenals and pancreas.

The interpretation of these findings points to a certain interrelation between endocrine function and regeneration. Liver cirrhosis is supposed to occur when the regenerative power of the liver is unable to cope with the repeated injuries. The important rôle of thyroid and gonad function in favoring regeneration generally has been proved experimentally. The pineal, as demonstrated by pathologic evidence, seems to have an inhibitory effect upon the gonads up to the age of adolescence. Destruction of the pineal in childhood leads to a development of secondary sexual characters and particularly to growth of hair on the body. Individuals with a persistent or hyperplastic pineal, such as is found in liver cirrhosis, are conspicuous for their hairlessness.

The theory has been brought forth that liver cirrhosis occurs only in such individuals whose constitution is anomalous. An underdevelopment of the thyroid and the gonads and persistency of the pineal inhibit the course of normal regeneration. Such anomalous individuals develop cirrhosis of the liver after chronic alcohol or other intoxications, while the livers of normal persons are capable of substituting the lost parenchyma through regeneration.

(Discussion by Dr. M. J. Stewart, Leeds, Eng.) There is a possibility which I think might be borne in mind; namely, that there are alcoholic drinks and alcoholic drinks. There is a striking difference between Scotland and England with regard to the incidence of cirrhosis. In eight years study in Scotland one saw very little cirrhosis of the liver. In England, in sixteen years one saw a great deal of it. A possible conclusion to be drawn from this is that the racial difference in incidence is due to racial difference in drinking habits. In England the stronger liquors drunk by the hospital class are gin and insufficiently matured whiskey. In Scotland, before the war, spirit drinkers, even among the poorer classes, drank only whiskey of good quality.

(Dr. M. A. Goldzieher, Budapest, closing.) This remark about the whiskey habit being prevalent in Scotland and the comparative rarity of liver cirrhosis seems to indicate that the Scotch have small pineals and good gonads and thyroids.

DECIDUAL CELLS IN BLOOD VESSELS. F. B. Mallory, Boston.

Abstract. In four cases of rupture of the uterus at a cornu as the result of interstitial pregnancy many of the blood vessels in the smooth muscle tissue were found to contain masses of decidual cells. This occurrence of decidual cells in

blood vessels during uterine and particularly during tubal pregnancy was observed as early as 1895 and several papers were written on the subject in the following ten years.

Study of decidual cells shows that they are formed in two ways, by transformation of proliferated fibroblasts, especially in the upper layer of the mucosa, and of existing fibroblasts, especially in the myometrium. The cell processes of the old fibroblasts retract and the fibroglia fibrils become wavy and corkscrew-like and in time disappear. The nuclei of these decidual cells often become multiple as a result of direct division forming giant cells which in the past have often been interpreted as syncytial cells that have invaded the uterus.

Evidently absorption of a chemical substance (a hormone) secreted by the chorionic epithelium stimulates the adjoining fibroblasts to proliferate and to change to decidual cells. Diffusion of this hormone from the uterus out through the oviducts may cause decidual formation in the mucosa of the tube and occasionally on the outside of the uterus.

Absorption of it around and within the blood vessels especially beneath the lining endothelium, may cause proliferation of the fibroblasts and transformation of them into decidual cells. In this way masses of them may be produced within blood vessels. Those in the veins may be carried away in the blood stream, those in the arteries are packed in the terminal branches of the vessels and occlude them.

In one case of chorionepithelioma of the uterus the tumor had invaded arteries and veins, but the numerous mitoses and the differentiation of Langhans' cells into syncytial cells rendered easy the recognition of the kind of cells in the blood vessels. In the second case the decision was more difficult. The tumor growth was slower and several arteries were packed full of decidual cells. In the myometrium were many large cells which might be either tumor cells or decidual cells, probably the former.

It is important to recognize the normal occurrence of decidual cells in the blood vessels of the oviducts and uterus during pregnancy, otherwise they may be mistaken for the tumor cells of chorionepithelioma.

(Discussion by Dr. Alfred Plaut, New York.) I wonder whether I was correct in understanding Dr. Mallory that in the so-called chorionic invasion there is no true chorionic invasion in uterine vessels but that these cells have to be considered decidual cells. Does this apply generally to all cases or to certain cases? Second, I should like to know whether there are substances which are responsible for the decidual change in tissue other than the endometrium, or whether absorption by lymph stream or the blood stream has to be considered. The decidual change in the cul-de-sac is found in a rather high percentage of all cases of pregnancy. I wonder if we always have to consider the way through the tube for this substance. Concerning the decidual change in connective tissue of the tube — a few years ago I saw a case like this one and I published it. I would not do it to-day because I have seen since that it is not so infrequent as I thought. It was more a direct change of the connective tissue cells of tubal folds than a multiplication of cells. It seemed that there must be some action of the decidual cells upon the covering epithelium because in looking through slides I was guided after a short experience by flattening of the epithelium and in the neighboring tissue found the decidual change. The swelling produced by decidual change was not sufficient to explain the flattening of the epithelium. Furthermore, edema which may give a picture simulating decidual change did not lead to this flattening of the epithelium.

(Discussion by Dr. V. C. Jacobson, Albany.) Occasionally in pregnant women one sees foci of decidual change in the tubal mucosa and in the ovary. Cases have been described in which there were multiple foci of decidual cells beneath the mesothelium of the pelvic viscera, including intestines, and without endometrial epithelium. The explanation of this phenomenon would seem to be that some hormone of pregnancy had acted on fibroblasts where it had come into contact with them, converting them into decidual cells in the same manner as Dr. Mallory postulates for his findings in and about uterine blood vessels. And another point of importance from Dr. Mallory's observations is that his identification of the cells found in the uterine vessels in some cases of chorionic epithelioma as decidual cells and not tumor cells, probably explains the good operative results in many cases, the cells which metastasized being benign decidual cells and not tumor elements.

(Discussion by Dr. M. A. Goldzieher, Budapest.) Dr. Mallory's paper is of great importance in those cases which have been described as chorionepithelioma in males. I believe that some of these tumors may be derived from teratomas and may really be classed with chorionepitheliomas. The majority of these, however, should be classed with giant cellular angiosarcomas and they are certainly altogether different from chorionepithelioma. The development of such decidual-like cells from endothelial cells is much more plausible and I think Dr. Mallory's work supports this point. I think that the formation of decidual cells is not always dependent upon the influence of hormones because in the cases of male chorionepitheliomas, there is no evidence of any hormone activity.

(Dr. F. B. Mallory, Boston, closing.) The observation of something which caused decidual cells to appear in the lymph nodes near the uterus has been made in the past and is well known. Another explanation of how decidual cells are formed beneath the mesothelium is stretching of the uterus so as to allow the direct escape of something which causes the decidual cells to form.

MYOCARDIAL CHANGES IN CARDIAC DEFECTS. Maude E. Abbott, Montreal.

Abstract. Very little has been done to determine the histologic appearances of the myocardium in cardiac anomalies, although there is quite a rich literature on the inflammatory and degenerative changes that may occur in the myocardium of infants as a result of congenital cardiac syphilis. The most important studies are by Letulle (Presse Medicale) who demonstrated a chronic pericarditis in a number of cases examined and von Zalka (Frankfurter Zeitschr., 1924). The latter author examined fourteen cases of cardiac anomalies, four of which were from his own necropsy service, and of which eight were to be classed from the gross appearances as purely developmental in origin (cardiac septal defects) while six showed a stenosis or atresia of one or other of the semilunar orifices, suggesting an inflammatory origin. No changes were noted by him in the first group of so-called developmental cases, but in the other six cases of congenital valvular lesions he found extensive myocardial changes, and inflammatory processes in four and recent in two.

In several cases which have come recently within the personal experience of the writer of pulmonary or aortic atresia the myocardium of the ventricle giving off the occluded vessel showed macroscopically large yellowish gray areas unmistakably evidencing disease and which microscopically showed degenerative and inflammatory lesions resembling those described by other writers. The corresponding character of these lesions and their relationship to the proliferated and myxomatous endocardium (which presents a curious resemblance

to the embryonic endocardial cushions) seemed to us of sufficient interest to warrant the presentation of two of these cases here. The appearances in these cases appear to us to suggest that primary myocardial disease, probably of syphilitic origin has caused arrest or improper involution and irregular fusion of the primitive bulbar cushions and a failure of the muscular septal bands of the ventricle to invade these and that this may be in some cases the cause of conus stenosis.

(No discussion.)

THE PATHOGENESIS OF OLD VALVULAR DEFECTS. B. J. Clawson and E. T. Bell, Minneapolis.

Abstract. In addition to the vegetations in acute endocarditis there is a diffuse inflammation, always in the free edge and often involving the greater part of the leaflet. This circumstance explains the uniform thickening so commonly seen in old defective valves.

Rheumatic vegetations are composed chiefly of fibroblasts, and in the process of healing they readily become converted into fibrous tissue. There is no ulceration and no organization. Fifty-five of seventy-three old defective valves are considered the result of rheumatic endocarditis, and, in twenty-seven of these, incompletely healed rheumatic lesions were recognizable.

Bacterial endocarditis is a more intense inflammation than the rheumatic. Proliferation predominates but exudation is often prominent. Large thrombi are formed on the raw surfaces and there is often ulceration. Healing consists in the conversion of the leaflet into scar tissue. Such portions of the thrombi as do not become detached persist indefinitely without becoming organized, although they may become calcified. Complete healing rarely occurs. Three of seventy-three old defective valves were interpreted as the result of bacterial endocarditis.

Transitions between rheumatic and bacterial vegetations are frequently seen. Rheumatic vegetations were found in association with bacterial in three-fourths of the cases of subacute bacterial endocarditis.

Fifteen of seventy-three old defective valves belong to the aortic calcified nodular group. The etiology of this type is unknown. There is no satisfactory evidence that it is of inflammatory origin, and it seems unrelated to atheroma. Aortic stenosis in the absence of disease of any other valve is usually of this form.

Stenosis is more frequent than insufficiency in old defective valves.

The only old pulmonary valve defects seen were of the congenital type (3 cases of pulmonary stenosis).

An acute rheumatic endocarditis may terminate in several different ways: (*a*) death during the acute stage from toxemia; (*b*) partial or complete healing followed after a variable interval by the reappearance of fresh rheumatic vegetations (recurrent rheumatic endocarditis); (*c*) partial or complete healing followed by the formation of bacterial vegetations on the valves — a more active inflammation (subacute bacterial endocarditis); (*d*) slow incomplete healing giving rise to deformed leaflets on which rheumatic inflammation is still recognizable; (*e*) complete healing resulting in thickened, stiffened valves with smooth surfaces.

As to pathogenesis, seventy-six hearts with old valvular defects are interpreted as follows: fifty-five from rheumatic endocarditis, three from bacterial endocarditis, fifteen (all aortic stenosis of the calcified nodular type) of undetermined origin, and three (pulmonary stenosis) congenital.

(Discussion by Dr. A. M. Pappenheimer, New York.) I wish to ask Dr. Bell whether he has frequently found transitions between the rheumatic and the bacterial types of endocarditis. We have recently observed a case in which the two types of valvular disease coexisted. On one surface of the mitral leaflets were frank bacterial vegetations, with colonies of micrococci and a profuse cellular reaction; on the opposite surface were small vegetations of the rheumatic type without demonstrable bacteria. The myocardium likewise showed two distinct types of lesion. In addition to focal necrosis and cellular infiltrations such as are usually found in bacterial endocarditis, there were characteristic Aschoff bodies. Thus, although the two types of infection may coëxist, and each in an active form, it has always seemed to us fairly simple to differentiate them histologically.

(Dr. E. T. Bell, Minneapolis, closing.) Sometimes one sees valves in which the rheumatic vegetation is on one leaflet and the bacterial on the other. As I mentioned, if you study all subacute cases and look over the leaflets very carefully, you will find rheumatic vegetations along with the bacterial in about three-fourths, and usually on the same leaflet. If one wants to say that infectious subacute endocarditis is a different infection from rheumatic, one must assume a rheumatic infection complicating three-fourths of the cases of subacute endocarditis.

SPECIFIC LESIONS OF PERIPHERAL BLOOD VESSELS IN RHEUMATISM. W. C. VonGlahn and A. M. Pappenheimer, New York.

Abstract. In ten of a series of forty-seven consecutive cases of rheumatic carditis, vascular lesions of a specific type were observed in the following situations: lungs, aortic valve, kidneys, perirenal and periadrenal adipose tissue, appendix epiploica of sigmoid colon, testis, pancreas and polyp of cecum. In the lungs, practically every small branch of the pulmonary artery was affected; in other situations only isolated vessels.

The lesions are characterized by exudation of fibrin into and about the vessel, by necrosis of the cellular elements of the vessel wall, and by a very characteristic cellular reaction in the surrounding tissue. These acute changes are followed by organization, often with the formation of new blood channels within the thickened intima, and occasionally in the media. Absence of thrombosis was a characteristic feature. The lesions differ from the commonly described forms of arteritis, and are regarded as of rheumatic origin.

(No discussion.)

MULTIPLE NECROSES OF SPLEEN (FEITIS) IN PERNICIOUS ANEMIA. O. T. Schultz and (by invitation) Norbert Enzer, Chicago.

Abstract. In 1921 Feitis described a condition which he termed Fleckmilz. The spleen in two cases of cardio-renal vascular disease was studded with grayish white nodules, varying in size from such as were just barely visible to others larger than a linseed. Microscopic examination proved these areas to be necroses which involved the Malpighian bodies. Since then a similar condition has been reported by Geipel and by Mathias, each of whom had seen a single instance in eclampsia, by Wilton in a case of pneumonia, and by Meuret who saw the condition in two cases of cardio-renal vascular disease.

A case of pernicious anemia, in a woman aged 59 years, is presented, in which the spleen contained multiple necrosis of the type described by Feitis.

The spleen was small, measuring 9 by 5 by 3 cm. On the cut surface were numerous grayish areas 1 to 2 mm. in diameter, which were believed to be hypertrophied Malpighian bodies. Microscopically these were areas of coagulation necrosis, each involving a Malpighian body. In the sinusoids were large phagocytic cells which contained fragments of nuclei and erythrocytes. These cells were especially numerous in the sinusoids immediately about the Malpighian bodies.

In the previously reported cases the necrosis of the Malpighian bodies began at the center and was due to occlusion of the arteries of the corpuscles. In the case here reported the necrosis began at the periphery of the body and was due to filling of the sinusoids of the peripheral zone of the Malpighian bodies by phagocytic reticulo-endothelial cells.

(Discussion by Dr. Alfred Plaut, New York City.) Did these large cells contain fat or lipoid?

(Dr. O. T. Schultz, Chicago, closing.) The cells in question did not contain lipoid. Although probably of the same origin, they were not identical with the large, lipoid-containing reticulo-endothelial cells which may be so numerous in the condition which Bloom has termed lipoid-histiocytosis. Their situation also differed in the two conditions. In lipoid-histiocytosis most of the cells are free in the pulp between the sinuses, whereas in the case herewith reported they were present within the sinuses. In the latter case they were filled with particulate matter derived from erythrocytes and leucocytes.

THE VASCULAR MECHANISM OF THE SPLEEN. W. L. Robinson, Toronto.

Abstract. In order to demonstrate the histologic structure of the spleen and the nature of the blood flow through it, sheep and dog spleens were injected via the arteries and veins with a variety of solutions. In all cases the detail structures were shown up quite readily by injecting the pulp to capacity through the vein with fixing solutions such as Zenker's and 10 per cent formalin. To demonstrate the nature of the arterial circulation a warm 25 per cent gelatine solution with or without carmine pigment was found the most satisfactory. Varying amounts of these solutions were injected with the object of grading the injections to determine the course of the fluid mass through the vascular and pulp systems. The gelatine *in situ* was satisfactorily stained with iron hematoxylin, providing that the differentiation was not carried too far. In the cases where carmine pigment was added to the gelatine, picric acid was used as a counterstain instead of picro-acid fuchsin mixture.

The arteries were found to occupy the central portions of the splenic lobules as outlined by Mall. Branches from these penetrated the lymphoid corpuscles of Malpighii and divided into a number of very fine branches of capillary size and structure supported by the pulp cells. In all cases they were found to terminate abruptly in a pear-shaped end-organ, the ellipsoid of Schweigger-Seidel. This was found to consist of a group of cells, endothelial in type and function, covering the end of the fine capillary branches and supported directly by the pulp cells. The portion of the capillary surrounded by these cells was quite permeable to the gelatine solution, slightly less to the carmine pigment. The surrounding ellipsoid cells, while appearing as compact masses, were found on injecting the gelatine solutions, to have intercellular spaces allowing for the flow of the injection mass from the ellipsoid capillary to the pulp and venous sinuses. The ellipsoids were found to have a very intimate relationship to the

pulp spaces and most of our injected material was found to flow directly into the pulp spaces. On the other hand the ellipsoids were found, in a number of cases, to be lying in direct contact with venous sinuses. Such close contact allowed a direct flow from ellipsoid through the intercellular spaces of the venous sinuses. These same venous sinuses were also found to have free communication with the pulp spaces by their slit-like stomas.

We are of the opinion that the vascular mechanism of the spleen is of the nature of an open circulation — the bulk of the blood flowing from ellipsoid through the pulp spaces to the vein. On the other hand much of the flow is more or less direct from the ellipsoid into the venous sinuses through their intercellular spaces.

(Discussion by Dr. H. T. Karsner, Cleveland.) The necessity for reproducing many of our pictures in black and white means very often that the beauty of the demonstration is obscured. I have had the privilege of seeing some of Dr. Robinson's preparations in his laboratory in Toronto and I wish to say that as convincing as this demonstration is in black and white, a view of the original preparations, or series of preparations, is much more so.

(Discussion by Dr. E. B. Krumbhaar, Philadelphia.) There have been numerous physiologic examples recently described where by means of shuntings, alternating activity or other expedients, the circulatory needs of an organ can be kept at a minimum for average purposes and yet quickly adapted to much greater needs. I need only instance Krogh's capillaries and Richards' work on the glomeruli of the frog's kidney. Dr. Barcroft's recent work on the spleen would indicate that such mechanism was peculiarly needed in the spleen. You may recall that he showed that there was very extensive reservoir power of the spleen for blood, whereby in response to demands, such as exercise, carbon monoxide poisoning, etc., very considerable amounts of blood could be brought out into the circulation. In a cat, for instance, with a very "muscular" spleen the number of corpuscles sent into the general circulation amounted to one-third of the total volume of the blood cells of the body. In some cases, as in carbon monoxide poisoning, where the hemoglobin carrying power was affected, the presence or absence of the spleen was shown to make the difference between life and death. It thus becomes a practical matter of some importance. It is extremely desirable, of course, if such is the case, to have as many ways of getting the blood circulating through the spleen as possible, and it is particularly pleasing to hear Dr. Robinson's demonstration showing that, as I understood him, blood can come through the "open" circulation of the pulp as well as by direct communication between the arteries and the small arterioles and the sinuses. Possibly Dr. Robinson is familiar with Braus' Anatomie der Menschen, recently published, in which he presents a diagram showing numerous ways in which the blood may get from the arterial to the venous system, by (1) direct outpouring from the arteries into the pulp, or (2) through the fenestrated ends of the arteries into the pulp, or (3) by direct communication of artery with the venous sinus. In the same way blood is taken up by the sinuses either through trumpet-shaped openings or through the sinus fenestrations. Such an arrangement, with its various controllable mechanisms for holding back or hastening the blood through the spleen, would fit in well with the functional observations previously referred to. I would like to ask what connection these ellipsoids of the arterial capillaries have with the Husenarterien and the penicillia of Ruysch.

(Discussion by Dr. E. M. Medlar, Madison.) This gives the structure of the histologic unit and is interesting. I wonder if the author has also studied the capillary distribution in the Malpighian corpuscles.

(Dr. W. L. Robinson, Toronto, closing.) At no point was I able to demonstrate a direct connection between the artery and vein. I know some believe that such occurs, but I think these can be explained by the presence of a vein running parallel to the ellipsoid and cut tangentially. At no point could I trace a thread of gelatin through to a vein. In the dog, I was of the opinion that the flow in all cases was through the ellipsoid. As far as the Malpighian bodies are concerned, I could demonstrate ellipsoids in many of them. Some claim that they are unable to inject the Malpighian bodies. In many cases I found that I could do it quite readily.

THE OPEN CIRCULATION OF THE SPLEEN PULP. Ward J. MacNeal, New York.

Abstract. In the study of spleens obtained at surgical operation or at necropsy, the gross examination is completed promptly. After taking a culture specimen by capillary pipette, a small portion of the organ is removed at one end, for preparation of smears and for fixation in the collapsed state. A cannula is next introduced into an arterial branch near the other end of the spleen and this is perfused with Locke's solution with addition of 1 per cent sodium citrate and 0.25 per cent gelatin; followed by a little salt solution and then Helly's formalin-Zenker fixing solution, so as to fix the spleen in a moderately distended state. Near the other end one makes a similar injection into a tributary of the splenic vein. There are thus available for study (1) smears, (2) sections of undistended spleen, (3) sections of spleen distended by artificial perfusion, (4) sections of spleen distended by venous perfusion.

In attempting to ascertain the connections between arterial and venous capillaries the nucleated erythrocytes of birds have been injected through arterial branches into very fresh human spleens and have been introduced through a gastric branch of the splenic artery into the living spleen of anesthetized animals, with subsequent precisely timed fixation.

The sections of distended human spleen have so far not revealed any recognizable direct continuity between arterial and venous capillaries. It is easy to recognize the terminations of arterial capillaries in the spleen pulp, especially in the marginal zone of the Malpighian corpuscles. The terminations are usually distended and the lumen communicates with the pulp spaces between protoplasmic strands of branched reticulo-endothelial cells. In the pulp cords between the venous sinuses, arterial capillaries are also found. They are longer than the others. They also terminate with an ampula in the pulp, but with very little substance intervening between the ampula and the adjacent venous sinuses, so that an injection mass might readily give the impression of direct continuity. From the pulp there are many minute openings into the venous sinuses, especially the smaller ones adjacent to the marginal zone of the Malpighian corpuscle.

The experimental injection of bird's corpuscles with subsequent prompt fixation has conclusively demonstrated that these corpuscles pass readily from the arteries into the spleen pulp, especially in the marginal zone and from this through the wall of the venous sinuses to gain the venous lumen.

The observations are in close agreement with the conception of Weidenreich and are distinctly opposed to the conclusions of Helly in regard to the circula-

tion of the spleen pulp. The blood passes here in spaces without defined vascular walls. The circulation is in this sense an open one.

(Discussion by Dr. L. U. Gardner, Saranac Lake.) May I ask either of the former speakers whether there is any information available as to the comparative structure of the spleen of the guinea-pig and the rabbit?

(Dr. W. J. MacNeal, New York, closing.) I have no information on guinea-pigs at all. We have undertaken to study a few guinea-pig spleens but none of the material is ready. So far I can speak only of human and rabbit spleens.

OBSERVATION OF FORMATION OF GIANT CELLS IN TURTLE BLOOD CULTURES. Mortimer Cohen (by invitation), Pittsburgh.

Abstract. Observation was carried out on hanging drop cultures of turtle blood. Some of the cultures were made in accordance with Lewis' method, others were vitally stained with neutral red and Janus green and in a few instances, blood from turtles which had been injected with India ink was used. Individual cells and groups of cells were studied for as long as twenty-six days. During the periods of greatest activity the microscopic fields were not changed and almost continuous observation was carried out. Quiescent mononuclear cells with undifferentiated protoplasm were observed to develop Brownian movement, rod-shaped mitochondria, to send out pseudopodia and to take on the characters of wandering cells. Multinucleated giant cells were observed to form by fusion of large mononuclear wandering cells. Nuclear division was not seen. No attempt was made to deterimne the nature of the cells which fused to form giant cells. In later cultures multinucleated giant cells were seen to give off multinucleated masses which continued to live for some days as individual multinucleated giant cells.

(No discussion.)

GIANT CELLS AND THEIR RELATION TO CASEATION IN TUBERCULOSIS. E. M. Medlar, Madison.

Abstract. From this study the "giant cell" in tuberculosis is found to be similarly produced in guinea-pig, fowl and man. The material which has been considered as its cytoplasm is in large part composed of necrotic tissue or of caseous exudate. In this dead material particles of reticulum can commonly be demonstrated. The "giant cell" is formed by mononuclear leucocytes wandering into and surrounding the area of necrotic exudate. The compactness of the dead substance apparently determines the arrangement of these cells and the consequent arrangement of the nuclei. Evidence of these cells wandering into the "giant cell" can be demonstrated. Particles of coal pigment in "giant cells" have been found. This demonstrates that mononuclear leucocytes which have previously phagocytized coal pigment at times participate in "giant cell" formation.

Lymphocytes have also been found within "giant cells."

It is apparent that "giant cell" formation follows rather than precedes areas of caseation or of necrosis. The mononuclear leucocytes and to a less extent the lymphocytes invade or surround these "foreign bodies" for the purpose of removing them by phagocytosis or by digestion from the tissue and thus promote complete repair.

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Abstract. This study shows the similarity of the process of caseation in the guinea-pig experimentally infected and in fowl and human hosts naturally infected. The primary reaction in all of these hosts to the tubercle bacillus is the formation of the mononuclear or "epithelioid" tubercle without giant cell formation, caseation or polymorphonuclear leucocytic infiltration. Lymphocytes play but little part in this phase.

If the resistance of the host is high and the dosage or the virulence of the tubercle bacillus low, hyperplastic tuberculosis results. This may go on to healing and fibrosis without necrosis, caseation or giant cell production intervening. In this reastion the polymorphonuclear leucocyte plays no part.

Following either an injury to the mononuclear leucocytes, or the production of a substance or substances by the growth or by the disintegration of the tubercle bacillus, the polymorphonuclear leucocyte is attracted. These cells accumulate in relatively small or in large numbers. For some reason they are early injured and die without the production of a typical abscess. These dead cells form the bulk of the caseous material.

At this stage, if sufficient autolysis has occurred and if opportunity for extrusion of the exudate is afforded, cavitation or ulceration ensues. If this does not occur, mononuclear leucocytes and lymphocytes wander into the caseous mass and attempt digestion and removal of the material. If the area be small, scar tissue may be the end result. If the area be large, calcification may be the end result. If the tubercle bacilli are not destroyed the process outlined above will occur in adjacent tissue with a consequent tissue destruction and spread of infection.

After an area of caseation is produced the polymorphonuclear leucocyte does not appear to be further attracted.

(No discussion.)

HEALING IN INTESTINAL TUBERCULOSIS. Leroy U. Gardner, Saranac Lake.

Abstract. The following observations are based upon a study of necropsy material from sixty-one cases dying usually from far advanced phythisis. Of this number, 46 or 75.4 per cent showed some evidence of intestinal involvement; of the 46 cases, 18 or 39.1 per cent showed gross or microscopic evidence of healing. The degree of healing, with a few exceptions, corresponds to the duration of treatment with the quartz mercury vapor lamp. Eleven cases receiving no heliotherapy were also studied. In two of these there was evidence of healing.

The process of healing consists in the arrest of the specific tuberculous foci or their destruction in the course of ulceration. This is followed by the development of an inflammatory granulation tissue in the floor of the ulcer which resolves without excessive scar formation. The denuded surface is covered by epithelium which regenerates from the border of the affected area. Occasionally where the destruction of tissue has been excessive, teat-like mammillations are found, sometimes singly and sometimes in clusters which persist apparently for an indefinite period to mark the site of former reaction. Such healing is accomplished with the development of stenosis from cicatricial contracture or of adenomatous growths. The only gross deformities produced are variable degrees of thinning and dilatation of the intestinal wall from destruction of the muscular coats and the previously mentioned mammillations.

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(Discussion by Dr. M. J. Stewart, Leeds, Eng.) I would like to ask what happens to the muscularis mucosae in healed ulcers. To what extent does deep penetration by glands occur?

(Dr. L. U. Gardner, Saranac Lake, closing.) In reference to the point of destruction of muscularis, there is apparently no attempt to regenerate smooth muscle cells. A break in the muscularis is filled in by granulation tissue which ultimately remains. In regard to deep penetration by glands — yes, penetration goes down to the outer third of the submucosa but I have not observed it to go any further.

THE HISTOPATHOLOGY OF THE SUBCUTANEOUS LESIONS IN TULAREMIA. Howard H. Permar and Grover C. Weil, Pittsburgh.

Abstract. The materials forming the basis of this paper were obtained by biopsy from a woman of 45 years admitted to the Mercy Hospital, Pittsburgh, Pa., in the tenth week of the disease. The history and clinical course were typical and the diagnosis was confirmed by agglutination tests. The available lesions consisted of subcutaneous nodules located along the lymphatics of the arm.

The tissue changes are typical of a granuloma and may be summarized in sequence as follows. The earliest reaction consists of massing of endothelial leucocytes with giant cell formation. The local capillaries show endothelial hyperplasia with narrowing and obliteration of their lumina. As a result of the narrowing and occlusion of capillaries, areas of bland necrosis are formed, involving the endothelial and giant cell collections. Bacterial toxins may be an added factor here. Many polymorphonuclear leucocytes migrate to the necrotic focus which goes on to liquefaction. The adjoining fixed tissues give rise to a cellular granulation tissue in which fibroblasts predominate while capillaries are rather few and narrow. Endothelial hyperplasia also occurs in the new formed capillaries. Secondary lesions appear in the inflammatory wall and these go through the same stages, leading to the formation of necrotic foci which tend to fuse with the primary one. In the meantime, the entire lesion is gradually surrounded by a zone of lymphocytes and plasma cells. The healing process is one of slow organization.

We have had an opportunity to study sections of inflamed axillary lymph nodes from two additional cases of tularemia in Pittsburgh. Both showed tissue reactions essentially the same as those we have described. The literature contains no adequate presentation of human tularemia, though Councilman and Strong in detailing the acute lesions in animals, noted fundamental reactions identical with those we have described in the human. The granulomatous character is seemingly not evident in animals since in them tularemia is an acutely fatal infection.

(Discussion by Dr. G. C. Weil, Pittsburgh.) It seems to be the prevailing impression that surgical intervention in the treatment of tularemia is rather a dangerous procedure. However, several of the lesions in this case were attended by rapid healing following incision and drainage as compared with the other lesions in which no drainage was established. We were rather impressed also by healing by primary union of the lesions which were totally resected and without further recurrence. Marked improvement of the condition of the patient and the healing of the wounds was noted upon administration of potassium iodide and Fowler's solution.

(Discussion by Dr. W. L. Holman, Toronto.) I had an opportunity of seeing in San Francisco in the Public Health Laboratory a good many animals with

tularemia and with plague. The technician there, when he looked at the spleen of an infected animal, said the tularemia could be told very rapidly by just feeling the spleen. It felt granular, as if little grains of sand were scattered through the spleen. In the case of plague the infected areas are soft and abscessed. It is therefore very interesting that this lesion is a granuloma which would confirm this observation of the technician who had been working with plague about eighteen years and had used this rather simple method of differentiating. It is also interesting that these cases in the eastern part of the continent practically all come from rabbits. I do not think there is a human case from the original ground squirrels, in which the infection was first found by Dr. McCoy. It is particularly interesting that we have not found human beings infected from the ground squirrel.

(Dr. H. H. Permar, Pittsburgh, closing.) I think I have nothing particular to add. The course of the disease clinically would lead one to expect a granulomatous reaction. Our patient was ill for two and a half months and has just left the hospital. Some of the English workers who became infected in the Lister Institute were ill for as long as a year and a half.

SUPRAVITAL STAINING OF CULTURES OF LYMPH NODE AND LIVER ENDOTHELIUM. Frank A. McJunkin, St. Louis.

Abstract. In an investigation of the origin of the mononuclear phagocytes of the tissues and blood the reaction to neutral red of lymph node and liver endothelia growing *in vitro* has been determined. One of the mononuclear phagocytes is the peroxydase reacting monocyte of Naegeli which is found in normal human blood. The term has incorrectly been applied to cells that do not give the peroxydase reaction. Sabin, Doan and Cunningham were the first to observe and describe a second variety of phagocytes which they encountered in examining rabbit peritoneal exudates. It is a non-peroxydase reacting cell. On supravital staining with neutral red it is characterized by an accumulation in the cytoplasm of dye granules in the form of a rosette. The author found the "rosette" cell to be present in great abundance in the juice expressed from lymph nodes. In lymph nodes stained supravitally, fixed in Zenker-formol and sectioned, the rosette cells were found to be the reticulo-endothelium. A third type of phagocyte which has little affinity for neutral red was demonstrated in rabbit peritoneal exudates by the author. To study further the reactions of the phagocytes to neutral red the lymph nodes and the liver of rabbits were grown in tissue cultures.

The groin lymph nodes of normal rabbits and of rabbits injected subcutaneously with India ink were grown in the plasma of the same rabbit. Besides the lymphocytes, there appeared in the cultures two larger cells, the fibroblasts and the reticulo-endothelial cells. When treated supravitally with neutral red the former showed only a few scattered neutral red granules in contrast with the latter which accumulated the dye in eccentric spherical masses, or rosettes. The reticulo-endothelial cells phagocytize the carbon which is situated in the cytoplasm outside the rosette. The fibroblasts contain no carbon or only a few small carbon particles.

The liver of rabbits injected intravenously with India ink gave outgrowths into the plasma clot which consisted of two types of cells, the fibroblasts and the reticulo-endothelium. When treated with dilute solutions of neutral red the fibroblasts were observed to react with the accumulation of a few scattered dye granules and the reticulo-endothelium reacted not at all or with the forma-

tion of neutral red granules scattered irregularly throughout the cytoplasm. In the older cultures the neutral red granules became quite abundant in the reticulo-endothelial cells but there was no tendency for them to be grouped as a mass or rosette. The reticulo-endothelial cells contained much carbon. In both lymph nodes and liver cultures the reticulo-endothelial cells were round or elongated and connected by cytoplasmic processes which differed from the multiple tapering branches of the fibroblasts. They have more the appearance of the processes connecting the reticulo-endothelial cells of the lymph node sinuses or the attenuated prolongation of the endothelial cells of granulation tissue. Repeated attempts to obtain growths of normal liver failed.

Conclusions. 1. By the method of supravital staining with neutral red there are two kinds of endothelium: the lymph vascular type that accumulates the dye in the form of an eccentric mass or rosette and the blood vascular type that does not react at all to the dye or reacts with the formation of a diffuse granulation.

2. The individual phagocytes that separate from these two varieties of endothelium appear to retain the characteristics of the parent tissues.

3. The two endothelial phagocytes are non-peroxydase-reacting cells and should not be confused with the monocyte of splenomyelogenous origin.

(No discussion.)

METHYLENE AZURE B IN STAINING SECTIONS OF HEMATOPOIETIC TISSUES. Ward J. MacNeal and (by invitation) Sadao Otoni, New York.

Abstract. When methylene azure B (trimethyl thionin) was first prepared in our laboratory it seemed to possess no special advantage as a stain over the methylene azure A mixed with methylene blue. The tests then made related especially to its use as a blood stain in alcoholic solution according to the technic of Leishman.

Since that time methods have been perfected for the preparation of trimethyl thionin in comparatively large amounts and we have had opportunity to try this substance in the staining of tissue sections, with highly satisfactory results in sections of spleen and bone marrow. In combination with phloxine or with eosin following fixation in Zenker's or Helly's fluid, it gives an extremely crisp differentiation of the nucleus and of various protoplasmic granules and the hemoglobin content of various cells is distinct. It has proven to be particularly serviceable for the differentiation of neutrophilic granules, for which purpose it is much superior to anything else we have tried.

The technic is as follows. The tissues are fixed, best by perfusion through the arteries, with Zenker's solution or with the formolized Zenker solution of Helly, followed by immersion in the fixing solution for 2 to 6 hours. Wash in running water 48 hours. Embed in paraffin and cut at 2 to 5 microns. Float the sections on water and fix to slide or coverglass with albumen fixative. Dry thoroughly overnight at 37 C to insure adhesion of the section. Carry the section through xylol and graded alcohols to water, then to very dilute Lugol's solution for 15 minutes; wash and place in decinormal sodium thiosulfate for 5 minutes and wash in water. This removes precipitated mercury from the tissue. Stain in 1 per cent phloxine or eosin ten to sixty minutes; wash and stain in 1:1000 azure B bromide with gentle agitation for one-half to five minutes until the section is blue. Wash in water and carry through graded alcohols to 95 per cent alcohol containing a little colophonium, where the section is allowed to bleach to the desired tint; then to absolute alcohol, xylol and balsam.

Somewhat better differentiation may usually be obtained by the technic described by Bensley for the granule staining in the islands of Langerhans. after staining with the azure B the excess of stain is removed carefully with filter paper without washing; dehydrate in acetone; then to toluol; then differentiate in absolute alcohol one part, oil of cloves three parts; then toluol and balsam.

(No discussion.)

HYPERGLYCEMIA AS RESULT OF VITAL STAINING WITH CERTAIN DYES. THE RELATION OF THE MICROSCOPIC DISTRIBUTION OF THE DYE TO THE BLOOD SUGAR LEVEL. Isolde T. Zeckwer, Boston.

Abstract. In a study of the factors concerned in the production of hyperglycemia, the method of vital staining was employed. Three dyes were found to cause a marked fluctuation in the sugar content of the blood: methylene blue, neutral red and safranin. After intraperitoneal injections of these dyes, there is a rapid rise of the blood sugar up to 200 to 300 per cent over the orginal value attaining a maximum in one to three hours, and declining to normal in about eight hours. Such response was invariable in rabbits, guinea-pigs, cats and dogs.

The immediate symptoms after injection of these dyes are flushing of the vessels of the ears, increased force of the heart, heightened blood pressure and increased force of respiration without evident asphyxia. Repeated injections of neutral red into the same animal cause no difference in the immediate reaction and no permanent increase in the blood sugar level. After a time nutrition is interfered with, the animals losing weight although they continue to eat well.

The dyes, after injection, are rapidly absorbed into the blood stream and stain diffusely all the tissues. They are eliminated into the lumen of the stomach and small intestine, and differ in this respect from other dyes studied, which were not eliminated by this route and failed to affect the blood sugar level. Methylene blue on account of its reduction to the colorless form, is difficult to trace histologically in individual cells, but the microscopic distribution of neutral red can be clearly seen. Aside from its presence in the organs to which all foreign material is carried, it is characterized by appearing in granular precipitate form in cells forming proteolytic enzymes, namely acinar cells of the pancreas and epithelial cells of stomach and small intestine. This seems to be of significance in view of the fact that it is known that neutral red and safranin combine chemically *in vitro* with proteolytic enzymes, forming a colored precipitate which contains all the enzyme. Methylene blue also was found to give a precipitate with trypsin *in vitro*, while the dyes not affecting the blood sugar level gave no such precipitate.

Epstein, in his recent work tending to show that trypsin neutralizes insulin, found that safranin when injected directly into the pancreas failed to cause an increase in blood sugar while all other substances so injected caused hyperglycemia. He accounts for this finding by suggesting that safranin fixes the enzyme within the cells and thus prevents the escape of trypsin into the blood stream, which according to his hypothesis would neutralize insulin. In the present experiments in which the vital staining of the entire animal is produced, the blood sugar curve is quite similar to the curve of hyperglycemia following injections of trypsin into the unstained animal, and the rapidity of the rise and fall in blood sugar, and the attendent circulatory phenomena would indicate that the

effect is one of increased glycogenolysis by way of a nervous mechanism rather than by failure in utilization of glucose due to neutralization of insulin.

These dyes appear then to have two actions: (1) to combine chemically with intracellular proteolytic enzymes and (2) to cause hyperglycemia apparently by way of the autonomic nervous system. That these two actions of the dyes bear any relation to each other seems probable but remains to be proved.

(No discussion.)

HYALINE DEGENERATION OF ARTERIOLES AND CAPILLARIES: EXPERIMENTAL PRODUCTION IN ANIMALS. Herbert U. Williams and (by invitation) P. T. McIlroy, Buffalo.

Abstract. Hyaline degeneration of the arteries of the lymph nodules of the spleen may be found in practically every human subject 35 to 40 years old. In cases of arteriosclerotic kidney, hyaline degeneration of the interlobular arteries is usually present, which makes this degeneration of great practical importance. Hyaline degeneration of the arteries of the spleen nodules is also frequent in children from the fifth year on, and is seen in connection with a great variety of infections and intoxications; small areas of necrosis in these nodules often appear in the same cases. The hyaline change may appear in arterioles of smallest size, practically capillaries, and the lumen may be greatly narrowed and even obliterated. Degenerative changes seem insufficient to account for the mass of new material that appears under the endothelium. Study of arteries in spleen nodules gives the impression that the new material may well be in large part some substance derived from blood plasma; the association of the condition with infections and intoxications, especially in children, suggests that an increase in the permeability of the intima may permit the passage of such a substance into the vessel wall. The experimental work of the writers has been done on cats. Hyaline masses, sometimes quite large but usually small, are frequent in the lymph nodules of the spleen of the normal cat, being present in five-sixths of the normal adult cats examined; they are absent in kittens. Often an arteriole or capillary may be seen near the center of the hyaline body; frequently a hyaline thickening of a capillary wall may be demonstrated. In attempting to produce similar changes in cats the writers have used chiefly arsenic trioxide and histamine. The former was selected in the hope that the arsenic, or products of its action on other tissues, might be shown to have injured the lining of the small vessels and thus to have increased their permeability. It was thought that histamine would lead to dilatation of capillaries and possibly injure the lining also, and thus promote exudation of some of the constituents of the plasma. Both agents were given hypodermically, sometimes only using one of the two in a certain animal, sometimes using the two in one animal at successive injections. In order to eliminate the possibility that hyaline masses might already be present, a bit of spleen was removed by operation and examined before beginning the injections. Very young cats were also used to some extent without preliminary operations, but they proved very refractory to the treatment. In general, in spite of many negative and doubtful results, it seems that either arsenic or histamine, but better arsenic followed by histamine, may produce hyaline masses associated with the walls of arterioles and capillaries in the spleen nodules; sometimes the picture seen in human spleens of hyaline degeneration of capillaries was reproduced. Frequently, the morphologic relations and appearance of the new material made it practically certain that

it originated as an exudate. Small areas of necrosis were sometimes observed and in such cases the picture was closely like that seen in such human infections as scarlet fever. No certain results were obtained in vessels of the spleen that could definitely be called "arteries."

(Discussion by Dr. W. J. MacNeal, New York.) I am interested in this. I can confirm the occurrence of changes of this kind in the small arterial vessels of the spleen in very young children. In one such case the child had a severe hemorrhagic purpura with very low count of blood platelets. Following the splenectomy there was definite clinical improvement and increase in the platelets of the circulating blood.

(Discussion by Dr. H. T. Karsner, Cleveland.) In some work with Hanzlik several years ago, we pointed out that if a sufficient time elapse following injections of histamine, platelets are clumped in large masses in the pulmonary capillaries. It is at least conceivable that some of the masses along the capillary walls and vascular spaces may be clumped platelets.

(Discussion by Dr. Alfred Plaut, New York.) I would like to know about the reaction of these masses. Was fibrin stain or Russell's hyalin stain used? I would call attention to a few slides of mine which are on exhibition outside and which show changes related to the ones described in Dr. Pappenheimer's paper this morning.

(Dr. H. U. Williams, Buffalo, closing.) We have not tried the fibrin stain on every one of these, but tried it on quite a lot. There was no suggestion of the morphology of fibrin in any of them. I doubt if Dr. Karsner's suggestion as to thrombi of platelets will help. We had thrombosis in mind, and we considered various other explanations and have given them all up. I will keep that in mind, Dr. Karsner, and am obliged for the suggestion.

EXPERIMENTAL EMBOLIC GLOMERULONEPHRITIS. B. J. Clawson, Minneapolis.

Abstract. Fourteen rabbits were injected in the left ventricular cavity from one to five times with finely ground agar which had been heavily seeded with streptococcus viridans. Sixteen rabbits were similarly injected with agglutinated streptococci. Glomerular injuries were produced similar to those found in human kidneys in cases of subacute bacterial endocarditis.

(Discussion by Dr. H. T. Karsner, Cleveland.) The importance of this contribution is great and the features I wish to suggest are to be regarded in the light of constructive criticism. Successful experiments are reported with agar infiltrated with streptococci and we have no information as to what happens if sterile agar be injected. The paucity of lesions in those animals into which streptococci clumped by agglutinating serum were injected, raises the question as to whether or not streptococci clumped by other methods, as for example, acid agglutination, might not produce more frequent lesions of the glomeruli. It is at least possible that the agglutinating serum may so injure the organisms as to inhibit their pathogenic capacity.

(Dr. B. J. Clawson, Minneapolis, closing.) I should have mentioned that controls are run with sterile agar alone and so far no lesions have been produced. I think that agglutination produced by any method would give as good results as we have here. What we need to have is the organism stopping within the glomeruli. If you can get the organism to stop in the glomeruli they will set up a proliferation rather than an exudation. Any type of agglutination would serve, I should think, but I have not tried it.

HISTOLOGIC CHANGES IN EXOPHTHALMIC GOITER FOLLOWING THE ADMINISTRATION OF IODINE. Alfred S. Giordano, South Bend.

Abstract. This study was made from a group of sixty-eight thyroid glands removed from exophthalmic goiter patients, who had received iodine before operation. As a basis of comparison, the author used glands taken from patients who had died following a typical exophthalmic goiter crisis. The study reveals very striking involution changes from an active hypertrophy and hyperplasia to a colloid type of goiter. On reviewing the clinical histories of these patients and comparing the histologic picture of the respective glands it was found that the degree and extent of involution was closely paralleled by and corresponded to the clinical improvement of the symptoms. The changes are similar in character as those described following ligation of the thyroid vessels, but occur rather uniformly throughout the gland.

(No discussion.)

ARTICLES READ BY TITLE

A SATISFACTORY METHOD FOR THE STAINING OF THE NERVE FIBERS OF THE IRIS. M. Balado (by invitation), Rochester, Minn.

Abstract. 1. Fixation: The eyeball is fixed in 10 per cent formalin for twenty-four hours. Transverse section of the eyeball in two parts, anterior and posterior. Detachment of the choroid from the sclera to the point of attachment of the ciliary muscle. With care detach all the choroid and the ring of the ciliary muscle from the sclera, and as the choroid and iris have been previously fixed they conserve their shape perfectly. This procedure has been described by Czermak in 1885.

2. Embedding: The embedding is made in gelatin. The iris is washed in water for 24 hours; then placed in 10 per cent gelatin for 24 hours; then 30 per cent gelatin for 24 hours. Subsequently the iris is placed in a little pasteboard box, filled with 30 per cent gelatin, and is allowed to freeze in the ice box. When the gelatin has hardened, detach the box, and place the gelatin embedded iris in 10 per cent formalin for 24 hours.

3. Sections: The frozen sections are made in the usual manner. The sections must be parallel to the anterior surface of the iris, because in that manner it is possible to obtain all the thickness of the iris in a very few sections. The sections are fastened to the slides by allowing them to dry in position, and then at a particular time, before the drying has gone too far, they are passed rapidly with a glass rod, through celloidin, covering the surface of the sections completely. The sections are then placed in 70 per cent alcohol for fifteen minutes.

4. Depigmentation: The modifications to the primitive Alfieri method are very numerous (Seligmann). We used the following technic: place the section in potassium permanganate, 1:500, for 24 hours. Then rapidly wash in water, and pass into 1 per cent oxalic acid. These operations are repeated as many times as is necessary, until the sections appear white. The time that the sections must remain in the oxalic acid depends upon the previous action of the permanganate. After 24 hours in this agent, 20 minutes in oxalic acid are sufficient. If the sections remain yellow or brown, all the operations must be repeated.

5. Neutralization: When the sections are white, they are passed into a concentrated solution of potassium alum (10 per cent), where they remain until they appear transparent.

6. Staining the nerve fibers: The sections are placed in a solution of 2.5 per cent ferric ammonium sulfate, C. P. (violet crystals) for 2 hours, then rapidly washed in water and passed to the hematoxylin of Heidenhain where they remain 24 hours. After this time the sections are again placed in the solution of ferric ammonium sulfate, C.P., for the differentiation of the medullate nerve fibers from the other parts of the iris. When the differentiation is completed, thoroughly wash in water, then in alcohol 70 per cent, alcohol 95 per cent, carbol xylol, xylol and Canada balsam.

A Case of Adiposis Dolorosa ("Dercum's Disease") with Necropsy. N. C. Foot, Cincinnati.

Abstract. This report deals with a very obese negress of sixty, who weighed over 350 lbs. and was brought to the hospital after a "stroke," unable to answer any questions. She was so fat that physical examination was totally unsatisfactory, beyond establishing the fact that she was covered with great fat-pads that did not involve the head, forearms, hands, legs (below the knee) or feet. No history of painful fat was obtained; had the patient been able to answer questions she might have enlightened us. She died, apparently from uremia, after several epileptiform seizures, less than twenty-four hours after admission.

Necropsy revealed the adipose condition, nephritis and cardiac hypertrophy and dilatation, as the immediate cause of death, and changes in most of the endocrine organs. The pituitary was enlarged and showed adenomatous hyperplasia and sclerosis; the thyroid was also sclerotic and enlarged; the suprarenals were hyperplastic and each presented an adenoma; the ovaries were atrophic and sclerotic; there was persistent thymic tissue, and there was a malignant tumor of the tentorium and a smaller one embracing the stalk of the hypophysis, both of them composed of very primitive cells corresponding in shape and arrangement with those of the dural endothelioma.

The case is discussed from the standpoint of the literature on adiposis dolorosa (Dercum's disease) and it is concluded that this is not a clinical entity, but a syndrome brought about by a variety of causes influencing the hypophysis primarily and the other endocrine organs secondarily, as Cushing has already pointed out.

Filtration with "Oiled" Filters. W. L. Holman, Toronto.

Abstract. While testing filter candles by the method reported by Krock and Holman it was noted that one of the candles showed air bubbles escaping at one spot under a pressure very much below that found in a previous test. This candle, I believe, had accidentally come in contact with a little paraffin on the laboratory table.

A number of filter candles have since been studied and after obtaining estimates of pressure for air leakage, average pore size, capillary pressure, rates of flow and time for cultures of bacteria to pass through, they were artificially treated with a mixture of petrolatum and paraffin oil.

The rate of flow after this treatment was decidedly reduced. Air bubbles also came through under much lower pressure. The Bechhold formula for average pore size would indicate that the pore spaces after they are partly filled with petrolatum and paraffin oil are larger than before treatment. I therefore agree with Mudd in giving more value to the rate of filtration than to such methods for establishing the size of the pore spaces.

A twenty-four hour culture of *B. prodigiosus*, grown at room temperature, was used to test the permeability of the filters and it was found that these bacteria pass through the treated candles in a shorter time and are found in a smaller amount of filtrate than in the untreated candles. These same filters after having the petrolatum mixture removed with xylol showed a more rapid flow of fluid and became less permeable for bacteria. It would appear that the treatment with petrolatum and paraffin oil reduces the absorptive character of the candles and also the size of the filter spaces. It is to be hoped that "oiled" filters may be found useful in reducing the loss by absorption of valuable constituents of filtered materials such as the antitoxins, viruses, enzymes and similar substances.

There is also a chance of error where a filter candle has been accidentally brought in contact with petrolatum or other oily material. Autoclaving does not remove such a substance but rather distributes it over the intergranular surfaces and prolonged treatment with xylol, or other active solvent for petrolatum, is necessary to clean effectively such a candle.

COMPARISON OF THE CHANGES IN BLOOD SUGAR FOLLOWING INJECTIONS OF FILTRATES OF B. PARATYPHOSUS B AND OF HISTAMINE. Maud L. Menten and (by invitation) Helen M. Krugh, Pittsburgh.

Abstract. With single injections of sublethal amounts of histamine, varying degrees of hyperglycemia were obtained, but a fatal hyperglycemia, of a secondary antemortem hyperglycemia similar to those found with paratyphoid B filtrates was not noted. Single lethal injections gave either no change in blood sugar or an immediate hyperglycemia of 250 to 300 mgm. per 100 cc. of blood.

Repeated injections of gradually increasing dosage increased the animal's resistance so that little or no hyperglycemia response to histamine was elicited with each successive injection. A typical anaphylactic rise of blood sugar occurred on injection of histamine in animals previously sensitized to *B. paratyphosus B* filtrates.

COMPARISON OF THE CHANGES IN BLOOD SUGAR PRODUCED BY INJECTIONS OF WITTE'S PEPTONE AND OF FILTRATES OF B. PARATYPHOSUS B. Maud L. Menten and (by invitation) Helen M. Manning, Pittsburgh.

Abstract. Five samples of Witte's peptone, following single injections, caused varying degrees of hyperglycemia but only one of these was capable of producing a hypoglycemia comparable to that obtained with filtrates of *B. paratyphosus B* in rabbits. We were not able to reproduce with peptone, filtrate curves consisting of an initial hyperglycemia followed by an interval lasting from several hours to a week, at the end of which time the animal died with a hyperglycemia. An injection of peptone repeated within forty-eight hours after the first injection gave a fatal hypoglycemia in three to five hours with one sample of peptone. A typical anaphylactic rise of blood sugar was obtained by an injection of Witte's peptone in rabbits previously sensitized to paratyphoid B filtrates.

SOME UNUSUAL FINDINGS IN THE VERMIFORM APPENDIX. Alfred Plaut, New York.

Abstract. 1. Encapsulated plant material in serosa of appendix. The patient S. S., 52 years old, Russian, had typhoid fever at the age of 12 and an abscess in the right axilla following it. In the year before admission to the hospital she had different attacks of pain in the right side. Four months before admis-

sion there was an attack of pain in the right lower quadrant, nausea, vomiting and fever (101 F). She was five days in bed.

Physical examination showed tenderness over the gall bladder region and in the right lower quadrant.

At operation a third degree retroversion was found with firm pelvic adhesions. The appendix showed nothing pathologic.

Gross specimen: Thin, slightly hyperemic appendix, 4.5 cm. long. In the serosa near the tip several glassy gray spherical cyst-like formations are found, the size of a millet seed.

Microscopic: The appendix is obliterated. The glassy foci in the serosa are onion-shell formations with plant material in their center. The plant cells are prismatic. They fully resemble cells from wheat seeds. The nuclei of these cells cannot be seen. This foreign material is surrounded by many thick layers of mostly hyalinized connective tissue. Occasionally one solid hyaline mass surrounds the plant material. In some of the nodules large giant cells are found, small ones in others and there are many without giant cells. In the nodules, large and small connective tissue cells are situated between the foreign bodies and the hyaline capsule. All nodules are in the serosa, none in the muscular coat.

Comment. Apparently this plant material came from the intestinal tract. Whether the perforation dates back to the typhoid fever forty years ago or took place only four months ago when the patient had an attack of appendicitis is difficult to decide. Neither explanation is easy to accept. Similar cases are on record observed after perforation of gastric ulcer and after traumatic rupture of cecum. But there is also one instance of peritonitis around plant material in a man 75 years old where the history gave no evidence of perforation.

2. Calcified body in serosa of appendix. A woman 36 years old was operated for a gynecologic trouble. The appendix was retrocecal, adherent to within one inch of its tip and the tip was sharply angulated.

Microscopic. In the serosa a calcified mass is found. It measures 0.3 by 0.15 mm. Surrounded by thin connective tissue it is situated between the serosa itself and the external muscular coat. It is extremely brittle, and pressure upon the coverglass breaks it up into fragments. A structure can be seen under the oil immersion, which seems to be thin layers and some fine openings probably corresponding to stomas. The mass represents a shell or a part of one, which is empty. No inflammation is found in the surroundings.

The patient knew nothing of any previous illness.

3. Eggs of a parasite in wall of appendix. Canadian woman 35 years old; besides her gynecologic history there is a vague statement of appendiceal trouble several years ago. At operation an appendix was removed which was described as short, thick and juicy by the surgeon.

The gross specimen showed nothing particular but microscopically remnants of ova were found in the wall of appendix, partly surrounded by epithelioid cells. One calcified egg has a kind of polar spine. One slide contains a circumscribed fibrous mass which probably represents a scar following total destruction of an ovum. The blood picture of the patient was normal and careful repeated search for ova in feces and urine was negative.

4. Hyalinization of veins in appendix. The patient, 30 years old, white housewife, came to the hospital with symptoms pointing to tubal gestation. Operation revealed a ruptured tube surrounded by brownish bloody material.

Careful microscopic examination shows no signs of pregnancy. The tube wall is inflamed. The blood vessels of tube show nothing particular. A hyperemic appendix 8 cm. long was received with the tube. The periappendicitis which is present is the one usually found after abdominal hemorrhage. The mesoappendix is normal. In the serosa and submucosa of the appendix most of the veins are surrounded by several layers of spindle cells in a similar arrangement as is frequently found around foreign bodies. Inside of the elastica interna hyaline masses are found, partly encircling the whole lumen, partly only one third of it. This material becomes red with eosin, dark orange in the Van Gieson mixture, bright blue with Weigert's fibrin method, red with Russell's method. It gives no reaction for amyloid. The arteries in the appendix are normal. The small veins in the muscular coat are normal too.

The patient has no symptoms of any systemic disease. We cannot explain this disease of the veins of the appendix.

The first two cases indicate that perforation of some part of intestine may take place without stormy symptoms, perhaps even unnoticed.

The third case is probably another instance of Bilharziosis without symptoms.

The last case is submitted in order to get suggestions.